Weeds

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Weeds

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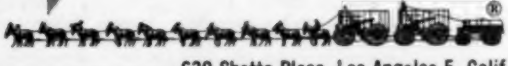

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Weeds

JOURNAL OF THE WEED SOCIETY OF AMERICA

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The Pantothenate-Synthesizing Enzyme, A Metabolic Site in the Herbicidal Action of Chlorinated Aliphatic Acids

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EVIDENCE that β-alanine or calcium pantothenate partially reversed the inhibitory action of several chlorinated aliphatic acids on yeast growth (4) suggested the hypothesis that synthesis of pantothenate is one of the metabolic pathways sensitive to this group of herbicides. The objectives of the experiments reported herein were (a) to determine the mechanism by which the enzymatic synthesis of pantothenate was inhibited by these compounds, (b) to seek evidence for the contributions of this mechanism, *in vivo*, to the herbicidal effects of 2,2–dichloropropionic acid (dalapon), (c) to determine whether pantothenate treatments could overcome the effects of dalapon in plants and (d) to test chloro-substituted analogs of pantoate for herbicidal activity. To achieve the last objective it was necessary to synthesize several new chemicals.

MATERIALS AND METHODS

Preparation and assay of enzyme.

Partially purified preparations of the pantothenate-synthesizing enzyme were obtained from acetone powders of *Escherichia coli*, ATCC 9637, by the procedure described by Novelli (9). Approximately 1 gm of acetone powder provided sufficient enzyme for each experiment. Enzyme activity was measured manometrically at 30° C by determination of acid-liberated CO_2 from pH 8 bicarbonate buffer under a 5-percent CO_2 atmosphere. The manometric technique was feasible because a net production of acid resulted from the overall reaction of synthesis of pantothenate from pantoate, β-alanine and adenosine triphosphate (ATP) (9).

Reagent solutions were adjusted to pH 8 with KOH prior to addition to Warburg vessels. The K pantoate solution was prepared from *l*-pantoyl lactone by the procedure of Maas (8). All solutions except ATP were mixed in the main compartment of the vessel

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during the equilibration period; ATP was added from a side arm to initiate the reaction. For routine analysis the composition of the final 3.0-ml reaction volume was 0.1 M KCl, 0.01 M $MgSO_4$, 0.01 M ATP, 0.02 M β-alanine, 0.02 M K pantoate, 0.066 M $KHCO_3$ and enzyme.

Herbicidal chemicals[3] used in enzyme studies were obtained as purified acids and solutions containing them were adjusted to pH 8 with KOH. The chemicals with their abbreviated designations and purities were as follows: 2,2–dichloropropionic acid (dalapon), 99+ %; 2,3–dichloroisobutyric acid (DCIB), 99+ %; trichloroacetic acid (TCA), USP; 2,2,3–trichloropropionic acid (2,2,3–TPA), 99+ %; and 2,3,3′–trichloroisobutyric acid (TCIB), 90% [impurities of 2,3,3–trichloroisobutyric and 2,3–dichloroisobutyric acids].

Liberation of CO_2 was proportional to enzyme concentrations up to rates in excess of 300 µl CO_2/hr. Greatest accuracy was obtained with enzyme concentrations which liberated about 250 µl CO_2/hr, and this quantity of enzyme was selected for subsequent investigations. ATPase, which occurred as a contaminate in all enzyme preparations, liberated an additional 20 to 60 µl CO_2/hr. Appropriate corrections were made for ATPase in all rate determinations. The ATPase activity was not affected by any of the chemicals used to inhibit the pantothenate-synthesizing enzyme. Enzyme-dissociation constants for substrate and inhibitory chemicals were determined for each of the individual enzyme preparations by the Lineweaver and Burk method of analysis (7) from the combined data of three independent experiments.

Seedling growth tests.

Barley (*Hordeum vulgare* var. Dayton, C. I. 9517) and oats (*Avena sativa* var. Clinton 59) were germinated and grown under greenhouse conditions in 8-oz polyethylene cups containing perlite saturated with Hoagland's solution. Moisture levels were maintained by surface applications of tap water. Barley seedlings were treated at the two-leaf stage, 9 days after planting. The tip of the first leaf was removed and the cut end of the leaf immersed in a vial containing 10 ml of a 0.02 M metabolite solution. Vials were removed 8 days later. The day following initiation of metabolite treatments, a sodium salt solution of dalapon was applied to the perlite surface of each cup in amounts equivalent to 4 lb/A, active ingredient (1.73 mg/cup). Plants were dissected 5 weeks after planting to determine the number of developed tillers that contained chlorophyll and were 1 cm or more in length.

Oat seeds (4 seeds/cup) were planted on a saturated perlite surface. The seeds were sprinkled uniformly with dry Ca pantothenate (0.1 gm/cup) and then covered with an additional 0.5 cm of perlite. Sodium dalapon was applied to the perlite surface in 10 ml of solu-

[3]The experimental compounds used in these studies were supplied by research personnel of the following organizations: Amchem Products, Inc., Ambler, Pa.; American Cyanamid Co., Stamford, Conn.; Dow Chemical Co., Midland, Michigan; Rohm and Haas Co., Philadelphia, Pa.

tion on the following day. Total fresh weight of tops from each cup was determined 3 weeks after planting.

Ryegrass germination tests.

Seeds of Italian ryegrass *(Lolium multiflorum)* were germinated in Petri dishes at 20° C. Fifty seeds were planted in each dish on three 9-cm disks of Whatman No. 2 filter paper, soaked for 1 hour in 10-ml solutions of chemicals. The molar concentrations reported for chemicals refer to concentrations of the 10-ml solutions. Excess solution was poured off at time of planting. Approximately 24 and 48 hours after planting all dishes were exposed to white fluorescent light for 15 min. This procedure resulted in more than 95 percent germination of the seeds. Seedling development was arrested by freezing after a 96-hour growth period. The lengths of the coleoptile and the root of each seedling were determined immediately after thawing of individual dishes.

Syntheses of new metabolic analogues.

2–chloro–4–hydroxy–3,3–dimethylbutyric acid, γ–lactone, *dl*–, (Table 4, II). The method was adapted from that of Darzens (1) with dependence on explanations by Gerrard (3). Into a 1-L reaction flask were put 100 gm (1.00 molar equivalent, *ME*) of *dl*–pantoyl lactone, 60 ml of (0.97 *ME*) pyridine, and 300 ml of anhydrous Et_2O. While this mixture was magnetically stirred and kept below 10° C with an ice bath, 96 gm (1.05 *ME*) of thionyl chloride dissolved in 100 ml of Et_2O was dropped in gradually over 1 hr, causing pyridine hydrochloride precipitation. The mixture stood overnight at room temperature with a ball-joint stopper acting to release pressure and prevent entrance of humid air. The Et_2O was distilled; then the warm system became a single-phase liquid that included the pyridine hydrochloride. The mixture was stirred at 130° for 15 min to disengage SO_2 from an intermediate considered to be *dl*-pantoyl lactone 2–0–chlorosulfinic ester. The transformation was completed during further stripping of volatiles with vacuum at this temperature, and by distillation at 122–140°/25 mm in a special still. To prevent obstruction of the still by keeping the condensate sufficiently melted, the distilled vapors passed directly to a receiver and then upward to a reflux condenser so that the receiver trapped reflux return at the condensation temperature. The distilled mixed solids, consisting of pyridine hydrochloride and product, were partitioned with water and benzene in a separatory funnel. The benzene layer was washed with water, dilute HCl, water, dilute equimolar proportions of $NaHCO_3$ and Na_2CO_3, and water; shaken with diatomaceous filter aid, anhydrous Na_2SO_4, and carbon; filtered; the solvent volatilized; and the product redistilled in the same still at 120–121°/14 mm. The yield was 84.5 gm, 74% of theory, of a colorless amorphous wax melting at 71.5° and having an aromatic odor of moderate intensity. Theory for $C_6H_9O_2Cl$, M. W. 148.60, Cl 23.86%, C 48.50%, H 6.11%; found Cl 23.77%, C 48.58%, H 5.74%. By infrared spectrum of the clear wax, pressed warm and solidified, the carbonyl

band center was at 1788 cm $^{-1}$ with maximum at 1790 (illustrating an α-chlorine shift from a corresponding 1775 center for *dl*-pantoyl lactone, pressed clear). Identifying multiplet maxima were at 1468, 1364, 1260, 1140, 1008, and 930; and a series of sharper peaks were at 901, 874, 844, 829, 798, 791, 692, and 660.

N–(2–chloro–4–hydroxy–3,3–dimethylbutyryl)–β–alanine, *dl*-, calcium salt (of acid IV, Table 4). The method was similiar to that published for calcium *D*(+)–pantothenate (12). With dry methanol to a volume of 50 ml, 1.03 gm (0.90 *ME*) of sodium was first dissolved, then 5.35 gm (1.2 *ME*) of β–alanine was partly dissolved by refluxing, and after cooling to room temperature, 7.43 gm (1.0 *ME*) of the lactone II was added. The mixture was machine-shaken, left 3 days at room temperature and filtered. The methanol was removed with vacuum below 30° C. The residue was shaken 1 hour with 2.7 gm (1.2 combining equivalents, *CE*) of anhydrous oxalic acid dissolved in 35 ml of absolute ethanol and filtered with diatomaceous filter aid. The filtrate was diluted with an equal volume of water, 6.6 gm (2.6 *CE*) of precipitated fine calcium carbonate added, shaken 1 hour with a pressure-releasing stack attached, and filtered. The filtrate was concentrated below 40° with vacuum, and the water more completely removed azeotropically by repeated similar concentrations after adding portions of 1:1 benzene and absolute ethanol. The concentrate was dissolved in a minimum of methanol, acetone was added until near precipitation, and the mixture was poured into acetone, giving a gummy precipitate. A specimen was manipulated between the surface of a ground-glass joint, allowing moderate heatless orientating seizures until hardening was evident; then scrapings of this were stirred into the main mass as crystalization seed. After crystalization had proceeded overnight, the lumps were crushed to a white powder, washed with acetone decantations, gathered on a filter, and air-dried; yielding 8.97 gm, 70% of theory, of a white powder that no longer seemed either hygroscopic or cohesive. Theory for $C_{18}H_{30}O_8N_2Cl_2Ca$, M. W. 513.45, Cl 13.81%, N 5.46%, Ca 7.81%. Found on a desiccated sample, M. P. 146–147°, Cl 13.14%, N 5.38%, Ca 7.55%. Mull spectra gave OH and/or NH bands at 3292 and 3094 cm $^{-1}$, and others at 1461–1414, 1370 and 1330, in hexachlorobutadiene; in Nujol the primary amide maxima were at 1661 and 1595, the ionized carbonyl maximum at 1550, and others at 1233, 1057, 1019, 988, 946, 914, 889, 815, 775, 682, and 657. For comparison Nujol mull bands of calcium *D*-pantothenate were found at 1653 (center of a 1659- and 1647-inflected amide-I type envelope) and 1560 (center of a broad band enveloping the amide-II and ionized carbonyl bands).

2–Chloro–4–hydroxy–3,3–dimethylbutyric acid, *dl*- (Table 4, VI). The lactone II was hydrolyzed in a Waring Mixer with conditions selected to make completion of hydrolysis evident by disappearance of turbidity; and special precautions were taken to diminish dehalogenation by alkali and to diminish re-lactonization by catalysts, heat, and dehydration. Near the final step an exposure to benzene was limited to a few minutes, because failure of a previous attempt was

attributed to re-lactonization within a few hours by benzene or associated catalysts. The final pure crystals were stored at low temperature and hastily used; the need for this was confirmed when the rate of reversion to the lactone was later determined and found expressable by a half-life of 43 days at 25° C.

The speed of a Waring Mixer was reduced with a variable transformer and its detachable chamber preheated in an oven to 100° C. In this was blended 300 ml of boiling water, 2 drops of a non-ionic surfactant (Triton X–100), 30 ml of ethanol, and 38.0 gm (1.00 *ME*) of the lactone II. After starting emulsification above the lactone M.P., ice was added until the temperature stabilized at 25°; and 20 gm of NaCl was added. Aqueous NaOH, 92.7 ml of 3.89 *N* (1.41 *ME*), was added and the blending continued for a total of 30 min or twice the time required for the solution to become nearly clear, as seen beneath the foam with the motor stopped. The excess alkali was then inactivated with 58.1 ml of 2.024 *N* (0.46 *ME*) HCl, theoretically leaving the product 5% acid-form and 95% neutralized. Diatomaceous filter aid and carbon were blended in and the solution filtered to give a clear neutral solution. To this in a separatory funnel were added 200 ml of Et_2O and 114 ml of 2.024 *N* (0.90 *ME*) HCl, the product account becoming 90% acid-form, 5% neutralized, and 5% removed. Three Et_2O extractions were made; each was washed with fresh saturated NaCl solution in a second funnel, and the used rinses were returned to the first funnel. The combined ether extracts were treated with anhydrous Na_2SO_4, filtered, and concentrated on a steam bath, without allowing the last of the ether to evaporate as a protection against heat exceeding 40°. Benzene, 20 ml, was added and the concentration continued with a 40° bath and vacuum to give a thick slurry of crystals. With haste to limit the benzene exposure the crystals were transferred to a filter and washed with four 20-ml portions of benzene, followed by five of petroleum ether, and then dried at 42°/50 mm for 30 min. The yield was 29 gm, 68% of theory, of odorless white crystals. Theory for $C_6H_{11}O_3Cl$, M. W. 166.61, and Cl 21.28%. Found Cl 21.28%, alkalimetric titration for the free acid 99.4%, ash below 0.04%, M.P. 90–91° (lactonizing influences probably could vary the M.P.) Nujol mull maxima were at 3400 (ROH); 2668, 2574, and 1709 (RCOOH); 1307, 1287, 1234, 1199, 1185, 1137, 1043, 980, 959, 923, 914, 825, 786, 720, 712, and 665 cm^{-1}. The band of lactone II at 1788, found a sensitive indication of reversion, only slightly modified the curvature.

After a specimen of the acid, VI, had reverted toward its γ–lactone, II, for 112 days at room temperature, and additionally for 30 min in a 90° C oven, for drying and melting to get spectrum of the clear wax, the infrared bands were found identical throughout the salt region with those of pure lactone II. This substantiated the intactness of the α–Cl in VI, which was not simply ascertained by a carbonyl frequency shift as for II, because in VI the γ–OH and COOH interactions exerted an influence opposite and approximately balancing that of α–Cl.

4–Chloro–2–hydroxy–3,3–dimethylbutyric acid, ethyl ester, *dl*-

(Table 4, I). The transformation of a γ-lactone to a γ–chloro ester was modeled after an example by Levene and Mori (6); they did not report the yield, and that here was small. With a solution of 13 gm (0.10 *ME*) of *dl*–pantoyl lactone in 46 gm of dry ethanol, saturation with HCl was approached by introducing 35 gm of dry gas. After standing 5 days at room temperature, stoppered with a ball joint as a safety precaution, it was poured into ice water and quickly extracted with Et_2O. That extraction, having scavenging stages, was not sufficiently discriminative; infrared indicated the residue was about 1:1 product and pantoyl lactone. The residue was redissolved in petroleum ether, washed with 4 water extractions, and distilled, giving at 86°/2 mm 1.8 gm, 9% of theory, of a colorless liquid. Theory for $C_8H_{15}O_3Cl$, M. W. 194.67, Cl 18.21%. Found, Cl 17.61%. Six foreign infrared bands corresponding to *dl*–pantoyl lactone diminished to insignificance during purification; therefore, from the chlorine content the product was concluded to be 96.7% pure, the remainder being *dl*–pantoyl lactone, which was tolerable for the purpose served. Characterizing maxima of the undiluted liquid were at 3500 (OH), 1729 (CO), 1472, 1451, 1393, 1372, 1273, 1217, 1119, 1084, 1018, 906, 863, 806, and 737 cm $^{-1}$.

RESULTS

Enzyme studies.

Inhibition of the enzymatic synthesis of pantothenate by dalapon was determined in combination with β–alanine by varying the substrate concentrations from 0.00167 M to 0.02 M. When these data were plotted graphically according to the method of Lineweaver and Burk (7) a family of parallel lines was obtained (Figure 1, upper). This type of inhibition, known as uncompetitive inhibition, indicated that the inhibitor did not couple with the enzyme at the point of β–alanine attachment. Data from a similar experiment testing for interaction between pantoate and dalapon gave a typical competitive inhibition test (Figure 1, lower). These data show that dalapon inhibited the enzymatic synthesis of pantothenate by competing wtih pantoate for a site on the enzyme.

A comparison of the enzyme-dissociation constants obtained with the individual enzyme preparations shows that dalapon and the natural substrate, pantoate, have approximately the same affinity for the enzyme. For three independent preparations of the enzyme the dalapon-enzyme dissociation constants (K_i) were 0.0021, 0.0025, and 0.0106 M. The corresponding dissociation constants (K_m) for pantoate were 0.0032, 0.0041, and 0.0060 M respectively. With a fourth preparation the K_m for pantoate was 0.0025. This variability in K_m among preparations is within the range reported previously (9).

Each of the five chlorinated aliphatic acid herbicides inhibited the pantothenate-synthesizing enzyme by competing with pantoate (Figure 2). The enzyme dissociation constants for the five herbicides were determined. In order of increasing affinity for the enzyme, the herbicides and K_i values were dalapon 0.0021 M, DCIB 0.0019 M,

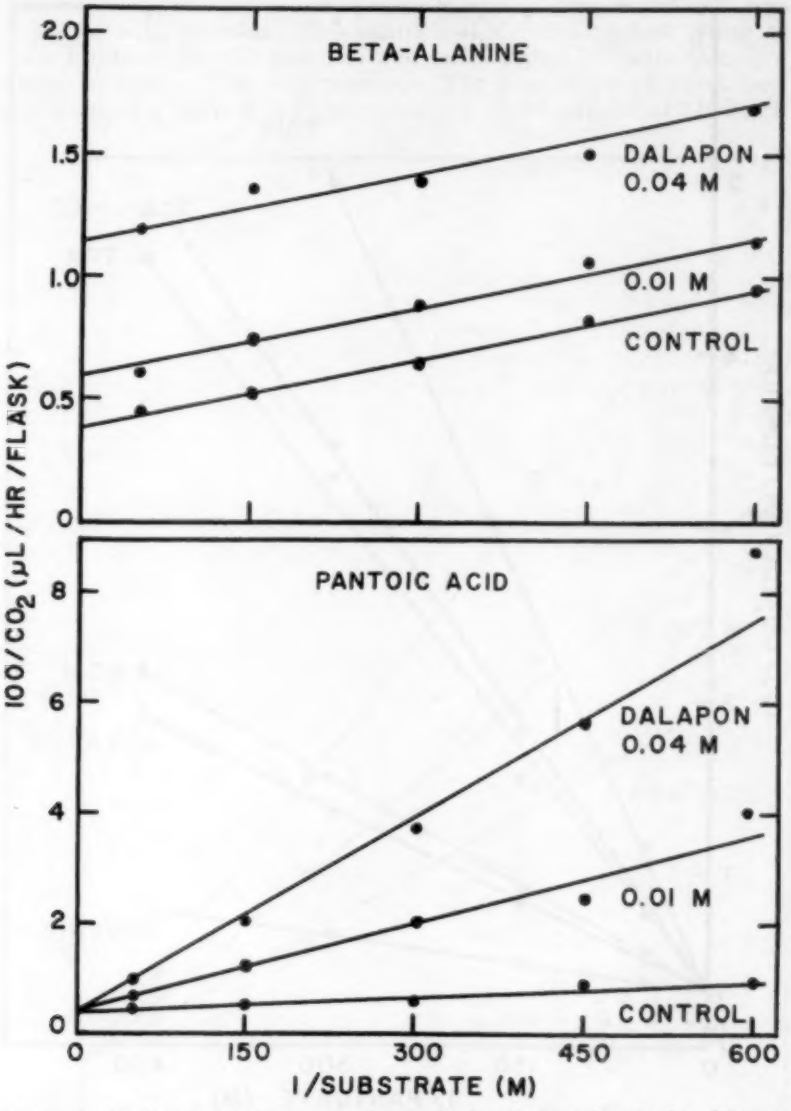

Figure 1. Inhibition of the enzymatic synthesis of pantothenate by dalapon shows the inhibitor to be uncompetitive with β–alanine and competitive with pantoate for a site on the enzyme.

2,2,3–TPA 0.0006 M, TCA 0.0006 M and TCIB 0.0003 M. The lowest constant represents the greatest toxicity. Therefore, the trichloro-compounds were obviously stronger inhibitors than the corresponding dichloro-compounds. The dissociation constant for pantoate

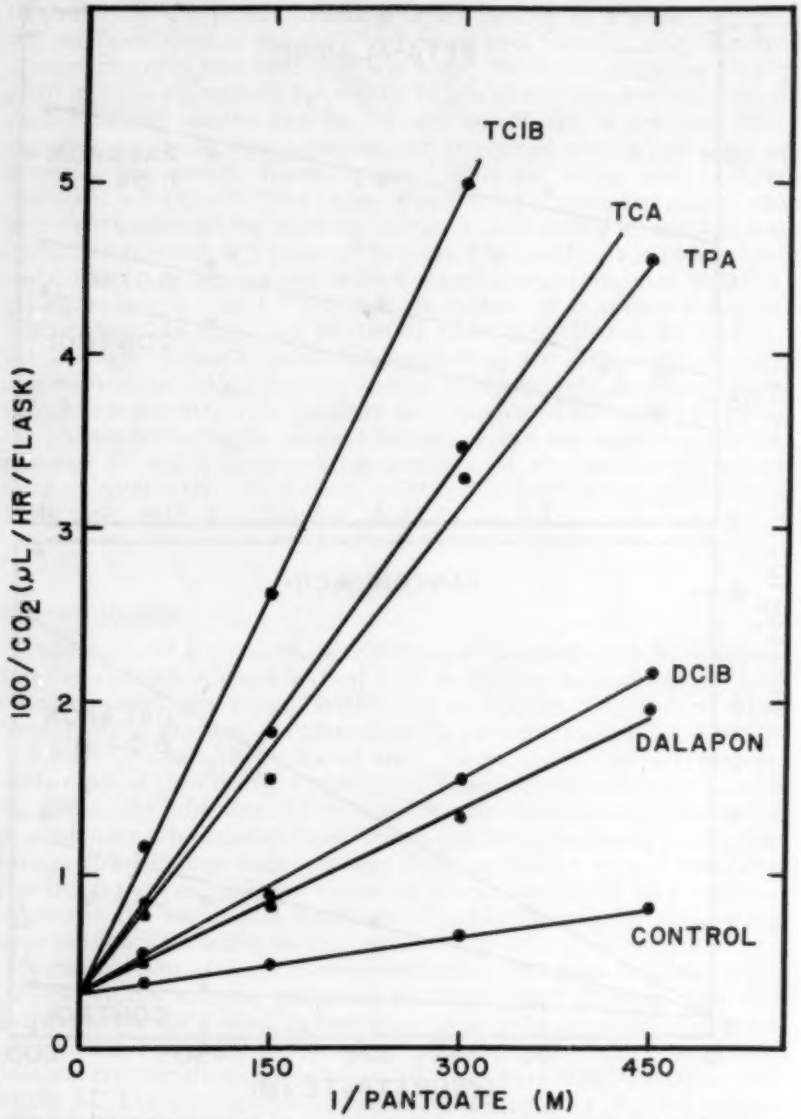

Figure 2. Competitive inhibition test for pantoate and five herbicides. Inhibitor concentrations were 0.005 M for 2,3,3′-trichloroisobutyrate (TCIB); TCA; 2,2,3-TPA; 2,3-dichloroisobutyrate (DCIB); and dalapon.

(K_m) on this preparation was 0.0032 M. Each of the herbicides had an affinity for the enzyme greater than the affinity of the natural substrate.

DL-2-chloro-4-hydroxy-3,3-dimethylbutyrate is an analog of

pantoate in which the α–hydroxyl group of the metabolite was replaced with chlorine. This compound did not replace pantoate as a substrate for the reaction and it inhibited pantothenate synthesis by competition with pantoate. The enzyme preparation used for the data presented in Figure 3 had a K_m for pantoate of 0.0060 M.

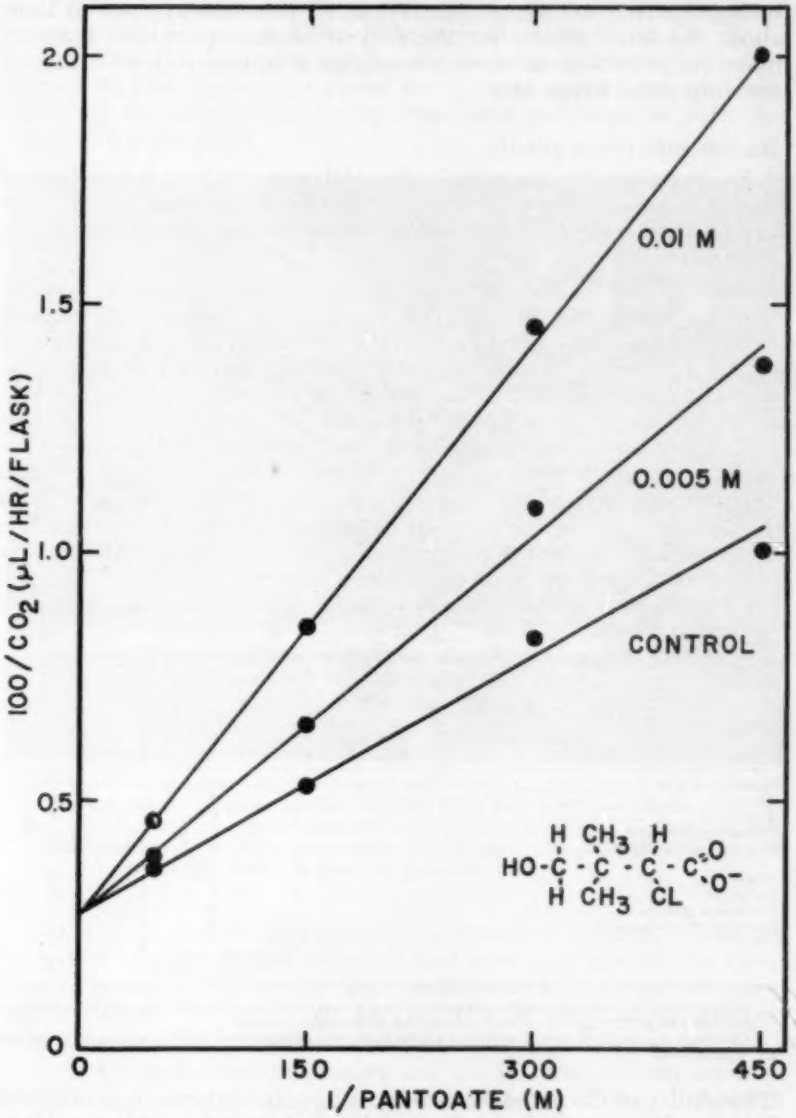

Figure 3. Competitive inhibition test for pantoate and the α-chloro analog of pantoate (structure shown).

On the same preparation the K_i values for dalapon and DCIB were 0.0106 and 0.0064 M, respectively; the K_i values for the pantoate analog calculated from the 0.005 and 0.01 M plots in Figure 3 were 0.0069 and 0.0084 M, respectively. Limited data obtained with a more sensitive enzyme preparation showed a K_i value for the analog slightly less than the K_m value. Therefore, the inhibitor obtained by substituting chlorine for the α–hydroxyl of *dl*–pantoate appears to have about the same affinity for the enzyme as does pantoate. Furthermore, its activity as an enzyme inhibitor is comparable with that of two important herbicides.

Studies with intact plants.

To examine the hypothesis that dalapon inhibits pantothenate synthesis *in vivo*, experiments were designed to determine whether any of the three metabolites could antagonize the effects of dalapon on growth of plants.

Barley. Both pantoate and pantothenate caused significant reduction in the abnormal tiller development on barley plants which resulted from application of sublethal concentrations of the sodium salt of dalapon (4 lb acid equivalent/acre). Untreated plants grown in the greenhouse during the winter developed an average of two tillers per plant in a 5-week growth period. Leaf treatment with water, β–alanine, K pantoate or Ca pantothenate did not alter the number of tillers developed; but plants grown in cups to which dalapon was applied to the perlite surface when seedlings were 10 days old had an average of eight developed tillers per plant 25 days after treatment. There was no evidence that β–alanine could antagonize the dalapon effect on tiller development; however, both pantoate and pantothenate reduced tiller counts significantly (Table 1).

Table 1. Antagonism between metabolites and dalapon as expressed by tiller development in barley.

Leaf treatment[a]	Dalapon[b] lb/A	Tillers per plant[c]
None	0	2.0
Water	0	2.1
β-alanine	0	1.8
Potassium pantoate	0	2.0
Calcium pantothenate	0	2.1
Water	4	7.8
β-alanine	4	8.0
Potassium pantoate	4	5.3
Calcium pantothenate	4	3.2
	LSD 5% level	1.86
	1% level	2.48

[a]Clipped leaf of 9-day-old seedlings immersed for 8 days in 10 ml of 0.02 M solution of metabolite.
[b]Applied to perlite surface of cups containing 10-day-old seedling.
[c]Average of a total of nine 5-week-old plants from two independent experiments with three and six replications.

The ability of Ca pantothenate to antagonize dalapon was observed in all instances. In other experiments, 0.01 M $CaCl_2$ could not replace Ca pantothenate as an antagonist in this test.

The tiller response in plants receiving the dalapon-pantoate combination of treatments was variable. The variation in response may have been due to insufficient uptake and translocation of pantoate in some plants. In all instances pantoate caused some damage to the leaf receiving the metabolite. The treated leaves developed chlorotic areas some distance above the surface of the metabolite solution and desiccation of leaf tissue often followed. In many plants the desiccated areas enlarged sufficiently to prevent further uptake of the metabolite. Pantoate damage to the treated leaf is apparent in Figure 4. This figure also shows the general appearance of plants receiving the dalapon-metabolite treatments as compared with that of an untreated plant.

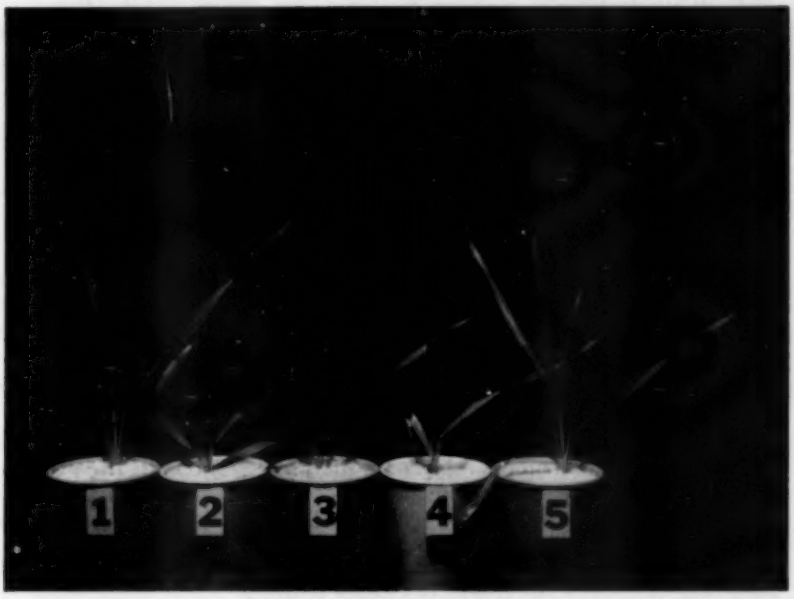

Figure 4. General appearance of 5-week-old barley plants treated with dalapon (4 lb/A applied to perlite surface 10 days after planting) and metabolites (cut leaf of 9-day-old seedlings immersed in 0.02 M solutions for 8 days). (1) Untreated control. (2) Dalapon plus water. (3) Dalapon plus β-alanine. (4) Dalapon plus K pantoate. (5) Dalapon plus Ca pantothenate.

None of the metabolites completely overcame dalapon inhibition of growth (Figure 4). Pantothenate had obviously altered the direction of growth of dalapon-treated plants, but fresh-weight measurements indicated little or no effectiveness in overcoming dalapon inhibition of total growth.

Oats. Ca pantothenate powder was placed over the oat seeds (4 seeds/cup) at the time of planting and the fresh weight of tops was determined 3 weeks later. The data in Table 2 represent the average fresh weight of tops per cup. Both winter and summer growing

conditions are represented in the growth periods of the three individual experiments. Analysis of variance, performed on the combined data, showed the interactions between Na dalapon and Ca pantothenate to be highly significant. Pantothenate alone did not affect growth of the young oat seedlings in the absence of dalapon; but pantothenate did partially offset the inhibitory effects of dalapon on growth of tops (Table 2). Considerable variation was observed

Table 2. Antagonism between calcium pantothenate and dalapon on growth of oats.

Dalapon[b] lb/A	Fresh weight of tops, gm[a]	
	Without pantothenate	With pantothenate[c]
0	2.7	2.6
2	1.74	2.48**
4	0.97	1.58**
LSD 5% level		0.330
1% level		0.437

[a]Average weight per cup. Each cup contained four 3-week-old plants. The data include three separate experiments with six replications each.
[b]Applied 24 hours after planting.
[c]0.1 gm/cup applied to seeds at time of planting.

among replications within individual experiments. This variability probably results from physical or chemical processes other than pantothenate metabolism which are involved in the plant response even at low dalapon concentrations. At higher dalapon concentrations (8 lb/A) very little growth was obtained even in plants receiving pantothenate. This illustrates further the inability of pantothenate to offset completely the toxic action of dalapon.

Ryegrass. The only evidence obtained for antagonism between dalapon and β–alanine in higher plants was observed in the growth of ryegrass seedlings. β–alanine at high concentrations was inhibitory to growth of both roots and coleoptiles (Table 3). Dalapon also

Table 3. Antagonism between β–alanine and dalapon as expressed by growth of ryegrass seedlings.

β-alanine concentration	Coleoptile growth, mm Dalapon conc.		Root growth, mm Dalapon conc.	
	0	0.001M	0	0.001 M
0	11.6	4.7	26.2	13.5
10^{-3}M	9.5	5.3	18.8	13.5
10^{-2}M	8.1	5.7	8.7	7.5

LSD's, 5% level of probability, based on data from 4x4 factorial experiment, 50 seeds per treatment: coleoptiles, 0.8 mm; roots 1.1 mm.

inhibited growth of both organs. However, when seeds were exposed to a combination of the two chemicals, the inhibitions were not additive and growth of coleoptiles was observed to be slightly greater than in dalapon alone. If the dalapon-inhibitory actions shown in Table 3 are expressed as percentage inhibitions of the corresponding

growth levels obtained with β–alanine alone, the inhibition caused by 0.001 M dalapon on coleoptiles was reduced from 60 percent to 44 percent to 30 percent and on roots from 49 percent to 28 percent to 12 percent at β–alanine concentrations of 0, 0.001, and 0.01 M, respectively. Such evidence for antagonism between the two chemicals suggests that both were affecting a single metabolic pathway. These effects might be expected if one assumes that β–alanine protects some sensitive growth site(s) against the inhibition by dalapon despite the fact that β–alanine is inhibitory.

Metabolic analogs.

The ryegrass germination-growth test was employed for a preliminary evaluation of the herbicidal activity of several pantoate and pantothenate analogs listed in Table 4. Four of the chemicals are new

Table 4. Toxicity of dalapon and metabolic analogs to root growth of ryegrass seedlings.

Number	Structure of chemical	Percent inhibition at conc. of	
		0.005 M	0.05 M
I	$Cl \cdot CH_2 \cdot C(CH_3)(CH_2OH) \cdot CH^a \cdot CO \cdot O \cdot CH_2 \cdot CH_3$	97	100
II	$CH_2 \cdot C(CH_3)(CH_2Cl) \cdot CH^a \cdot CO$, O bridging CH$_3$ and CO	77	100
III	$CH_3 \cdot C(Cl)_2 \cdot COOH$ (dalapon)	76	99
IV	$HOCH_2 \cdot C(CH_2Cl) \cdot CH^a \cdot CO \cdot NH \cdot CH_2 \cdot CH_2 \cdot COOH$	12	83
V	$HOCH_2 \cdot C(CH_3)(CH_2OH) \cdot CH^a \cdot CO \cdot NH \cdot CH_2 \cdot CH_2 \cdot SO_3H$	13	53
VI	$HOCH_2 \cdot C(CH_2Cl) \cdot CH^a \cdot COOH$	13	53

[a] *dl*-

compounds in which chlorine has replaced hydroxyl groups of the metabolites. Two of these chemicals, the ethyl ester of *dl*–4–chloro–2–hydroxy–3,3–dimethylbutyric acid (I) and the lactone of *dl*–2–chloro–4–hydroxy–3,3–dimethylbutyric acid (II), compare favorably with dalapon (III) as inhibitors of ryegrass root growth. A 50-percent

reduction in growth of ryegrass roots was obtained at 0.001 to 0.002 M for each of these three chemicals. The other two chloro-substituted analogs (IV and VI) have toxic properties as great as those of *dl*–pantoyl taurine (V), a commonly used antimetabolite of pantothenate. Percentage inhibitions of ryegrass coleoptile growth for the six chemicals agreed closely with the corresponding root-inhibition data recorded in Table 4.

DISCUSSION

The *in vitro* studies demonstrated that the chloro-substituted aliphatic acid herbicides inhibited the enzymatic synthesis of pantothenate by competing with pantoate for a site on the enzyme. The dissociation constants obtained for each of the six inhibitors indicated that the affinity of these compounds for the enzyme was about equal to, or greater than, the affinity of the competitive substrate. If this site and mechanism of action are involved, *in vivo*, in the plant growth responses effected by dalapon, then evidence for antagonism of dalapon action should be obtained by supplying either pantoate or pantothenate to the intact organism. Evidence for similar antagonisms by β–alanine might be expected in biological systems deficient in β–alanine, but not in organisms capable of synthesizing sufficient β–alanine to support optimum growth.

The evidence for interactions between dalapon and pantoate or pantothenate but not β–alanine in tiller development in barley was in agreement with the hypothesis that dalapon inhibited the enzymatic synthesis of pantothenate *in vivo* by the same mechanism observed *in vitro*. The inability of exogenous pantothenate to overcome completely the dalapon inhibition of total growth (fresh weight) of either oats or barley suggested that physical or chemical processes other than pantothenate-synthesis were probably involved in the mechanisms of action. Nevertheless, the evidence for a protective effect by pantothenate in the presence of sublethal concentrations of dalapon indicated that the enzyme of pantothenate-synthesis was probably one of the more important metabolic sites involved in the phytotoxic action of dalapon. A similar conclusion was reached in the earlier studies on dalapon inhibition of yeast growth (4).

Evidence for β–alanine protection of growth against the inhibitory action of dalapon has been observed with yeast, an organism incapable of synthesizing sufficient β–alanine to support maximum growth. The evidence for β–alanine–dalapon interactions in ryegrass suggested that a similar protective action by β–alanine may be functional in some higher plants. The enzyme data showing uncompetitive inhibition demonstrated that dalapon and β–alanine act independently at the enzyme level. However, the amount of product synthesized by the dalapon-inhibited enzyme was still dependent upon the degree to which the enzyme was saturated with β–alanine. When the enzyme is saturated with substrate a further increase in β–alanine concentration causes no measurable increase in pantothenate synthesis. This relationship is readily observed *in vitro;* and a similar relationship could be true *in vivo*. Therefore, evidence

for β–alanine antagonism of dalapon inhibitions of growth may be expected with organisms in which β–alanine is a growth-limiting factor. Lack of evidence for a β–alanine-protective effect in some organisms, therefore, does not negate the mechanism of action theory postulated from the *in vitro* studies.

Pantothenate treatments to overcome the phytotoxic effects of dalapon were partially effective in the experiments with barley and oats. Therefore, preliminary experiments (not reported) were initiated with pantothenate-treated seeds of corn, soybean, barley and oats to determine whether the metabolite could reduce dalapon damage to these crop plants. Dalapon was applied to soil containing pantothenate-treated seeds by the macro-screening technique described by Shaw and Swanson (10) for pre-emergence-herbicide-evaluation studies. These preliminary investigations were discontinued when evidence for pantothenate "protection" had been observed to be too inconsistent for the antagonisms to assume practical importance.

When an attempt was made to predict new chemicals which might limit plant growth by interfering with pantothenate metabolism, there came to mind the work on inositol and γ–hexachlorocyclohexane (5, 11). The chemical structure of the antimetabolite is identical with that of inositol except that each of the six hydroxyl groups of the metabolite is replaced with a chlorine atom. This led us to evaluate the phytotoxic properties of several new compounds in which chlorine was substituted for hydroxyl groups in the structure of pantoic acid. The tendency of γ-substituted butyric acids to revert to the lactone hampers the preparation of the analogs as purified acids. Nevertheless, a competitive enzyme inhibitor was obtained from the successful substitution of chlorine for the α–hydroxyl of pantoate. The effect of chloro-substitution on the γ–carbon of pantoate was not studied *in vitro,* but the ethyl ester of the γ–chloro-substituted analog possessed herbicidal activity on ryegrass which was comparable to the activity of dalapon. This analog is of particular interest since the γ–hydroxyl of pantoate is esterified with the pyrophosphate group in the coenzyme A molecule. The phytotoxicity of the four analogs suggests that other pantoate and pantothenate analogs should be evaluated for their herbicidal properties. The recent report that foliar sprays of sublethal concentrations of Na 2,3–dichloroisobutyrate prevent pollen development on cotton (2) suggests that the analogs might also be evaluated for plant "gametocide" activity.

SUMMARY

1. The hypothesis that the enzyme of pantothenate-synthesis is a metabolic site of dalapon action was confirmed *in vitro* and the mechanism of this action defined as competition by dalapon with pantoate for a site on the enzyme of synthesis. Similar results were obtained with several other chloro-substituted aliphatic acids.
2. Evidence for antagonisms between metabolites and dalapon on the growth response of plants treated with sublethal concentrations

of the herbicide is consistent with the mechanism described from *in vitro* studies and implicates the enzyme of pantothenate-synthesis as one of the important sites of dalapon action in plants.

3. Phytotoxicity was predicted for several new pantoate analogs in which the hydroxyl groups of the metabolite were replaced with chlorine. Four such compounds were synthesized and their herbicidal activities on ryegrass seedlings were subsequently demonstrated.

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Biological Assay of Herbicides for Fish Toxicity

JAMES T. DAVIS and W. S. HARDCASTLE[1]

INTRODUCTION

THE increasing use of toxic agents as pesticides for application contiguous to water areas has been viewed with trepidation by many fisheries workers. Herbicidal chemicals are viewed with particular alarm due to their increased use, both present and projected, for the control of aquatic vegetation where they are placed in direct contact with fish and other aquatic organisms.

In terrestrial weed control investigations where chemical applications are made on a standing crop, crop damage is usually reflected in stand or yield reductions which are directly measurable. Field evaluation of the effects of aquatic applications of chemicals on mobile animals such as fish and on the extremely small and inconspicuous benthos which make up the food chain upon which fish are dependent is much more difficult. This difficulty has led to the development of various laboratory methods for determining the effects of toxic agents on these animals.

As part of its over-all program for the control of aquatic vegetation, the Louisiana Wild Life and Fisheries Commission determines by bioassay the response of representative fish and benthic organisms to herbicides which may be used within the state. In conjunction with its stream pollution abatement program, other materials are also evaluated.

MATERIALS AND METHODS

Biological assay methods followed, generally, those outlined by Doudoroff, et al. (5), with temperature adjustments following those of Saila (12). Bluegill sunfish (*Lepomis macrochirus* Rafinesque) and largemouth bass (*Micropterus salmoides* Lacépéde) were used as test organisms. Both have been found to be medial in responsiveness when used as assay animals and are reasonably hardy and easily handled. More sensitive fish such as gizzard shad (*Dorosoma cepedianum* LeSueur) tend to respond very rapidly to toxic agents and fish such as bowfin (*Amia calva* Linnaeus) are so tolerant as to make them of little value for assay purposes. The species selected had the additional advantage of being sport fish common to fishing waters over a large area.

The following compounds were included: dimethylamine salt of 2,4–dichlorophenoxyacetic acid (2,4–D)[2]; isopropyl *N*–(3–chlorophenyl) carbamate (CIPC)[3]; hexachlorocyclopentadiene (C–56)[4];

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[2]Research sample supplied through the courtesy of Amchem Products, Inc., Ambler, Pennsylvania.

[3]Research sample supplied through the courtesy of Chipman Chemical Company, Bound Brook, New Jersey.

[4]Research sample supplied through the courtesy of Hooker Electrochemical Company, Niagara Falls, New York.

dimethylamine salt of 4–(2–methyl–4–chlorophenoxy) butyric acid [4–(MCPB)][2]; dimethylamine salt of 4–(2,4–dichlorophenoxy) butyric acid [4–(2,4–DB)][2]; 1,2–dibromo–3–chloropropane (Nemagon)[5]; 1,2–dibromoethane (EDB)[6]; mixed isomers of trichlorobenzoic acid, predominately 2,3,6–TBA, HC1281–S, (2,3,6–TBA)[7]; and mixed isomers of TBA, 2,3,5–trichlorobenzoic acid 16.3%, 2,3,6–trichlorobenzoic acid 7.8%, 2,3,5,6–tetrachlorobenzoic acid 36.4%, other isomers, 39.5%, ACP–M–323, (2,3,5–TBA)[2].

A median tolerance limit (TL_m) was established for each chemical. The notation TL_m as used by Doudoroff, et al. is essentially the inverse of the LD_{50} notation used in other biological investigations in that it indicates the concentration at which just 50 per cent of the test animals are able to survive for a specific exposure period. It is a notation specific to the evaluation of agents toxic to fish, while LD_{50} is a notation general to several biological evaluations.

At the beginning of the study, standard glass aquaria (20 x 12 x 10 in) were used as test vessels. These were found to be objectionable because of the extreme difficulty encountered in decontamination after the use of certain of the chemicals under investigation. They were also subject to breakage, and their size and shape made them space consuming in storage. After considering several possible substitute vessels, these aquaria were replaced with plastic-lined corrugated cardboard cartons which are manufactured commercially for milk transport.

The carton (Figure 1) measures 9 x 9 x 20 in, holds 25 L, and can be used repeatedly; the liner is of 4 mil pinhole free polyethylene and is discarded after being used for two determinations (turned inside-out after first use). The sides of the carton may be cut away to admit light; up to 60 per cent removal is possible with banding.

These cardboard aquaria have proven to be very economical, to occupy relatively little bench space, and to have essentially eliminated the decontamination problem; in addition, they are economical of storage space since they are stored folded and flat.

Since heterogeneity in water quality was expected to influence the reaction of at least some of the chemicals, water from two different sources was used. For each, pH, O_2 and CO_2 were determined and total solids analyses were made periodically during the testing period, January–December, 1957. Water for tests was obtained from the Ouachita River near Monroe, Louisiana and from Bayou DeSiard, also near Monroe, Louisiana.

Water in the aquaria was aerated by pressure fed stone airbreakers and was maintained at the saturation level during the course of each assay. Water temperature was held at 25° C ± 1° by the use of standard immersion aquarium heaters.

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[5]Research sample supplied through the courtesy of Shell Chemical Corporation, New York, New York.

[6]Research sample supplied through the courtesy of The Dow Chemical Company, Midland, Michigan.

[7]Research sample supplied through the courtesy of The Heyden Chemical Corporation, New York, New York.

Figure 1. Fiberboard cartons used as test vessels; as assembled for fish toxicity tests (left), as used to transport specimens (center), and with panel removed for light transmission (right).

Test fish were of fingerling size (3–5 in) from stocks available at the fish hatchery on the test site. Every effort was made to keep the size range for individual evaluations to a minimum. Ten specimens of a species were placed in each aquarium and were allowed 24 hr to recover from the shock of transfer.

After the fish had become acclimated, appropriate amounts of the test chemical were added to the 25 L of water to give the desired application rate. Where appropriate, concentrations were based on acid equivalent, otherwise, on active ingredient.

Fish in treated water were kept under observation for 1 hr after introduction of the test chemical to determine avoidance reaction, if any, and subsequent observations for this effect were made at 6 hr intervals.

For each chemical, trial concentration series were established using 1 aquarium and 10 fish for each concentration. Verifying series, consisting of 4 replicate aquaria, each with 10 fish, were subsequently used to validate TL_m obtained from the trial series. TL_m in each case was determined by graphic interpolation.

RESULTS AND DISCUSSION

Agricultural chemicals, particularly herbicides, are an important management tool for those interested in the development of water facilities for recreational activities such as fishing, boating, and swimming as well as for those interested in making water available for irrigation and commercial use.

Past histories of fish kills resulting from chemicals entering streams and lakes in run-off water or as drift from applications on crops point up the desirability of determining the toxicity of pesticides to fish and their food chain organisms, particularly where these materials are to be applied directly to water for the control of aquatic vegetation. While the literature is voluminous, it deals primarily with the effects of industrial effluents and is usually very general (4, 5, 6, 7, 8, 16, 17). Because of a lack of standardization in testing and in reporting results, information is often conflicting.

The method outlined by Doudoroff, et al. (5), acting as the Subcommittee on Toxicity, Section III, Committee on Research, Federation of Sewage and Industrial Wastes Association, has been accepted as the standard for the analysis of industrial wastes in water pollution control by the U. S. Public Health Service of the U. S. Department of Health, Education, and Welfare and by most other water pollution control workers. The use of the techniques to determine the effects of agricultural chemicals is in order since data so obtained have a common, standard base for comparison with existing toxicity data, and that to be obtained in the future by fisheries and pollution control workers.

In evaluating the toxic effects of pest control chemicals applied for aquatic vegetation control, the interaction of the various water constituents must be considered as influencing the activity of any given material. While a large amount of data from widespread locations will be necessary for final evaluation of this effect, water from two sources was used in this study.

The Ouachita is a dynamic river, subject to drastic seasonal fluctuation and influenced chemically by the release of commercial effluents and, consequently, subject to considerable variation in water quality. Bayou DeSiard is, by comparison, a quiescent cut-off of a flow stream, fed primarily by surface run-off and, therefore, subject to little fluctuation in water quality. The quality of Ouachita River water as indicated by the average of 12 monthly analyses and of Bayou DeSiard water analysed thrice yearly is shown in Table 1.

The response of bluegill sunfish and largemouth bass fingerlings to nine test compounds as indicated by TL_m in ppmw or ppmv, as appropriate, is shown in Table 2.

In tests by Applegate et al. (1) and by Springer (13) 2,4–dichlorophenoxyacetic acid and its various salts evidenced different toxicities to fish. In the present tests, the shift in TL_m from Bayou DeSiard water to Ouachita River water, while relatively small for the dimethylamine salt, was, nevertheless, present. While these TL_m concentrations are much higher than would be expected in any normal weed control operation, this variation also indicates the desirability of testing the toxicity of different formulations of the same chemical in waters of different quality where possible, particularly where various formulations are expected to be used in the field and where the TL_m and treatment rate have approximately the same value.

Applegate et al. (1) reported CIPC to be nontoxic to bluegill

Table 1. Quality of water from Bayou DeSiard and the Ouachita River as determined by analyses during 1957.

Determination	Bayou DeSiard[a,b]		Ouachita River[c,d]	
	Average	Range	Average	Range
pH	7.0	7.4– 6.6	6.7	7.3– 6.3
Dissolved O_2 ppm	7.3	7.7– 7.1	4.6	7.8– 1.3
CO_2 ppm	5.0	5.3– 4.6	4.0	5.6– 3.1
Hardness ppm	19.0	30.0–25.0	77.1	176.0–17.0
	Dissolved solids—ppm			
SiO_2	5.8	7.3– 5.0	7.0	12.0– 5.2
Fe	0.5	0.8– 0.3	0.2	0.3–Trace
Ca	4.0	Stable	16.3	48.0– 4.7
Mg	1.6	2.0– 1.4	4.3	13.0– 1.3
Na + K	10.5	11.0–10.3	67.9	244.0–18.0
HCO_3	0.0	—0—	16.7	23.0–10.0
SO_4	0.0	—0—	7.2	16.0– 3.6
Cl	5.2	Stable	131.2	468.0–32.0
F	0.0	—0—	0.4	0.6– 0.3
NO_3	2.5	3.6– 1.2	3.2	8.3– 0.2
Total dissolved solids	51.8	54.0–50.0	256.8	824.6–76.0

[a]Averages of 3 analyses.
[b]Analysis by Division of Water Pollution Control, Louisiana Wild Life and Fisheries Commission.
[c]Averages of 12 monthly analyses.
[d]Based in part on unpublished records of the U. S. Geological Survey.

Table 2. TL_m of largemouth bass and bluegill sunfish fingerlings to nine agricultural chemicals as determined by bioassay using water from two locations.

Chemical	TL_m ppm[a] in water from					
	Ouachita River		Bayou DeSiard			
	24 hr.		24 hr.		48 hr.	
	Sunfish	Bass	Sunfish	Bass	Sunfish	Bass
2,4-D	350	350	390	375	375	350
CIPC	10	8	20	15	12	10
C–56	—	—	[b]	[b]	30[c]	35[c]
4–(MCPB)	15	13	20	13	15	10
4–(2,4–DB)	10	10	20	15	8	10
EDB	18	15	25	25	18	15
Nemagon	50	30	125	50	20	20
2,3,6–TBA	1750	1300	1800	1500	1750	1250
2,3,5–TBA	150	90	175	125	90	55

[a]Averages of 5 repetitions.
[b]In excess of 500 ppm.
[c]C–56 tested at 96 hr gave TL_m of 25 ppm for sunfish and 20 ppm for bass

sunfish fingerlings at concentrations up to 5 ppm in Lake Huron water, however, no TL_m was determined. A large intraspecific difference in TL_m for this material in Bayou DeSiard and Ouachita River water was noted. Surber (14) reported no immediate danger to fish at 10 ppm with the related material isopropyl *N*–phenylcarbamate.

Applegate et al. (1) indicated that death can be expected very quickly when bluegill sunfish are exposed to C–56 at concentrations of 5 ppm; one sample resulted in death in 15 min while a sample from another source killed in 30 min. In tests with Bayou DeSiard water, both test species were in obvious distress within 5 to 10 min after the introduction of C–56; there was a loss of equilibrium and

only very slight response to touch stimulus. There were, however, no deaths in 24 hr at a concentration of 500 ppm and, consequently, no TL_m for this period was obtained.

4–(MCPB) is apparently much more toxic than 2–methyl–4–chlorophenoxyacetic acid, as reported by Hilliard.[8] Both fish species were noticeably agitated within 30 min after 4–(MCPB) was put into the test aquaria, and most specimens lost equilibrium within 6 hr at a concentration of 20 ppm. Results with 4–(2,4–DB) were similar to those with 4–(MCPB). 4–(2,4–DB) was more toxic than the dimethylamine salt of 2,4–D. However, the time lapse between loss of equilibrium and death was less than that observed in 4–(MCPB) tests. Apparently the substitution of the butyric for the acetic acid radical increases the toxic activity of these materials to bluegill sunfish and largemouth bass fingerlings.

In addition to the shift in TL_m between Bayou DeSiard and Ouachita River water, Nemagon increased markedly in toxicity as the time was extended. The TL_m for bluegill sunfish dropped from 125 ppm at 24 hr to 20 ppm at 48 hr.

The fish response to EDB was similar to the response to Nemagon, but not so drastic. Since this material is heavy and only slightly soluble in water, droplets were formed on the aquaria bottoms. Fish were seen to take these droplets orally, but no immediate ill effects were noted and time of death appeared to be independent of droplet ingestion.

Although slight differences in TL_m, both between test species in the same water and with the same species in different waters, were noted with 2,3,6–TBA, this material was of interest particularly for the early avoidance reaction of some of the test specimens in 2,3,6–TBA treated water. This response differed from the agitation previously noted in that the fish congregated at one side of the aquarium, away from the chemical, particularly where it was not evenly distributed when introduced. This type of avoidance reaction has led certain workers to advocate the use of strip treatments with some toxic materials on the assumption that fish would be repelled from the treated areas. The work of Jones (9) with phenolic compounds indicated the danger inherent in this assumption if complete toxicity data are not available. He reported avoidance at relatively high rates with no mortality, however, lower rates apparently blocked the sensory organs and the fish would remain in the toxicant.

The granular formulation of 2,3,5–TBA was much more toxic than was the liquid 2,3,6–TBA, and although consistent results were obtained in both Bayou DeSiard and Ouachita River water, the relatively large shift in TL_m for 24 and 48 hr is noteworthy.

It has been the practice of some investigators to report TL_m or comparable data from bioassays terminated at 24 hr or earlier. The activity of materials such as Nemagon, 2,3,5–TBA, and C–56 indicates the desirability of maintaining the tests for at least 48 hr, or

[8]Hilliard, D. R. The effects of herbicides and related compounds on fish. Master of Science Thesis n. p., Oregon State College, 98 p., 1952.

longer if possible. These data are of particular importance where full water treatment is to be used, or where relatively static water is to be treated, particularly if the use of a pelleted or stratifying material is anticipated.

Water quality has a direct bearing on the tolerance of fish to a given chemical and a probable effect on the concentration necessary for effective weed control. While the data here presented are far from conclusive in this respect, they indicate the advisability of considering water chemistry in future tests where at all possible. Only through a large number of observations will this particular facet of the aquatic weed control problem be brought into perspective.

SUMMARY

The response of bluegill sunfish and largemouth bass fingerlings to nine agricultural chemicals as determined by bioassay using water from two sources is presented.

With the exception of 4–(MCPB) tested against largemouth bass, the TL_m for test materials were lower in Ouachita River water than were comparable TL_m in Bayou DeSiard water, and for CIPC, the TL_m for 24 hr in Ouachita River water was lower than the TL_m for 48 hr in water from Bayou DeSiard. Several materials, such as 4–(2,4–DB), EDB, and Nemagon, evoked a decided shift in TL_m in the two waters. Other materials, such as 2,4–D, 2,3,6–TBA, and 2,3,5–TBA were less influenced by differences in water quality. There were minor interspecific differences in response of test fish, with bluegill sunfish generally being slightly more tolerant of the chemicals tested.

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Elimination of Rodent Cover Adjacent to Apple Trees

LeRoy Holm, Franklin A. Gilbert, and Ernest Haltvick[1]

The girdling of apple trees by field mice (*Microtus* spp.) is a common problem in most areas where this fruit is grown. Since these species of mice will seldom cross a bare area to feed, it is desirable to remove all vegetation at the base of the trees to help reduce the possibility of girdling. Many growers maintain such a bare area by slow and costly hand methods. Such methods are frequently employed in late fall, increasing the possibility that the newly exposed trunk area may incur winter injury.

The investigations in this report were initiated in 1953 to determine whether the ground cover around apple trees could be eliminated with organic herbicides. The most frequent and troublesome weeds in the experimental areas were *Agropyron repens* (quackgrass), and *Poa* spp. (bluegrasses).

It seemed reasonable to assume that woody, perennial plants with deep, well-established root systems, could withstand greater total amounts of herbicides than could young, herbaceous plants. The requirement that vegetation need only be destroyed in a narrow band adjacent to the trunk presented a second advantage in the treatment of mature trees since a major portion of the "feeder roots" are located at some distance from the trunk. A third advantage accrues for this type of treatment in the temperate zone if the herbicide is of a slightly soluble nature and tends to remain in the upper layers of the soil.

The purpose of these experiments was to test those organic herbicides which were effective against both broad leaved weeds and grasses and which were most resistant to breakdown in the soil. Early survey studies of various materials (1, 2, 3, 4) had shown that the urea herbicides were by far the most promising for this special purpose; this report will thus be confined to the use of 3–(*p*–chlorophenyl)–1,1–dimethylurea (monuron), and 3–(3,4–dichlorophenyl)–1,1–dimethylurea (diuron).

Materials and Methods

The experiments were designed to treat only the area at the base of the tree, since ordinary tillage and mowing equipment cannot eliminate all of the vegetation in this area. Conventional equipment may be used to obtain some degree of control of the vegetation in the row middles and under the drip of the branches.

There is at present no equipment which will reach under the low spreading branches of large trees to make accurate application of the chemicals in the restricted area of treatment. Therefore, a measured amount of herbicide was placed in 2 gallons of water in a sprinkling can and distributed over an area 8 feet in diameter at the

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base of each tree. With careful application, good distribution of the liquid in the area was obtained without mowing before application.

The nature of the problem was such that many small plots were established in order to obtain information regarding the response to monuron and diuron of trees of different varieties, ages, and orchard locations. The influence of single heavy applications and the cumulative effect of applications repeated annually were also evaluated.

Approximately 150 apple trees representing seven varieties of several ages were treated in 10 orchards in east central and southern Wisconsin. These orchards were on several types of soil ranging from sandy loam to heavy silt loam. During the years 1953–1957 extremes of both low and high rainfall and temperature were experienced in these areas. The fertility in all of the blocks was adequate for satisfactory tree growth.

RESULTS AND DISCUSSION

There was no evidence of injury from monuron or diuron on any of the 150 trees which were included in these studies (Tables 1, 2).

Table 1. Summary of treatments for six varieties of apples (varying ages) with monuron and diuron.

Treatment	Lb/A	Age of trees	Year	Variety and number of trees treated					
				McIntosh	Wealthy	Cortland	Delicious	Snow (Fameuse)	Va. Crab
Monuron	5	1	1955				1		
		4	1955				5		
	10	8	1955		5			5	
		20	1955	3		3	3	2	
		25+	1955	7					
		8	1956						5
		9	1956	5		5			
		20	1956			5			
		25+	1956	10	5				
		25+	1957	5	5				
Diuron	10	8	1956						5
		20	1956			5			
		25+	1956	10	3				
		25+	1957	5	3				
	20	25+	1956		1				
		25+	1957		3				
	30	25+	1956		1				
		25+	1957		3				
	40	25+	1956		1				
	60	25+	1957		3				

Table 2. Summary of repeated applications of monuron applied at the base of apple trees.

Variety	Number of trees	Age of trees	Monuron applied, lb/A, in					Total chemical applied lb/A
			1953	1954	1955	1956	1957	
Wealthy	5	25+	10	10	10	10		40
	5	25+	10		5	5	5	25
	5	25+	10		5		5	20
Seedlings	4	6		10	5			15
	4	6		5	2.5			7.5

In examining the trees for injury, particular attention was given to signs of discoloration on the margins of mature leaves. This is frequently the first, and is certainly the best documented, symptom of injury from urea herbicides in a wide range of plants. Other studies by the authors (1, 2, 4) have shown that in sour cherries, raspberries, and strawberries, leaf discoloration was the first sign of injury following the use of urea herbicides. Careful observations for effects on terminal growth, size of fruit, yields, and fruit quality were also made.

When applications of monuron were made in the early part of the growing season (last week in April), the first signs of yellowing of the quackgrass and bluegrass appeared in about 2 weeks. A complete kill had usually taken place in about 2 months. Diuron also eliminated all of the vegetation but the action was somewhat slower. In those areas treated with 10 lb/A the vegetation dried out completely by fall. The dead grass was then easily removed from the treated area by raking. In no instance in these tests has a single application of 10 lb/A of monuron or diuron failed to give complete control of vegetation for two full seasons (Figure 1). No other weed,

Figure 1. Elimination of vegetation beneath a mature apple tree by monuron at the close of the second season following a 10 lb/A treatment.

annual or perennial, has invaded these areas during this time. A rate of 5 lb/A of these herbicides has not cleaned up established quack stands.

The uncertainty of mouse populations and the value of mature apple trees make it somewhat difficult to combine this type of study with one in which trees are not protected by poisoning for the control of mice. However, in one orchard in which inadequate poisoning treatments were used, there was considerable girdling of trees in a 10 acre block. Ten trees in this area had been treated with monuron and diuron and as a result, the vegetation had been killed at the base of the trees. None of the trees treated with monuron and diuron were girdled. Elimination of the cover adjacent to apple trees will not replace conventional methods of baiting, etc., for mouse control. However, elimination of cover adjacent to trees will aid in limiting injury since the mice seldom feed where there is no protective cover.

SUMMARY

1. Monuron or diuron has been applied to areas adjacent to the trunks of apple trees at rates ranging from 2.5 to 60 lb/A. Some areas were treated with 10 lb/A annually for 4 years.

2. One hundred and fifty trees of varying ages, of seven varieties, and growing on light to heavy soils in 10 locations were treated. These trees experienced extremes of moisture and temperature.

3. Ten pounds per acre of monuron or diuron have given complete control of all vegetation for 2 to 3 years following application.

4. No sign of injury has been observed on any tree during the 5 years of this study.

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Evaluation of Herbicides for Several Species of Ornamentals[1]

S. K. Ries, B. H. Grigsby and H. Davidson[2]

One of the major costs in the production of woody ornamentals is for weed control by cultivation and hoeing. In nurseries, plants are grown in the same location for two to five years, and several species often are grown in mixed blocks. Thus, there is a need for a herbicide which is non-toxic to as many species as possible.

Sesone, NPA, CIPC, and mixtures of these materials have been shown by several research workers to be effective herbicides in nurseries (1, 3, 4, 5, 7). Recently, neburon also has proven to be a good herbicide for ornamentals (2, 8). Simazin has also shown promise as a herbicide for ornamentals (6, 9, 10).

The purpose of this study was to evaluate these herbicides and others, under various environmental conditions and with various species of ornamentals. This study had various phases which are discussed independently.

Screening Test on Lining-out Stock of Four Species

Materials and methods.

Two-year-old lining-out stock, 12 to 18 inches high, of *Cotoneaster acutifolia, Syringa chinensis, Taxus cuspidata (capitata)* and *Taxus media 'Hicksi'*, was transplanted on June 4 and 5, 1957, in a randomized block with three replications on a Hillsdale fine sandy loam. Individual treatments contained four plants of cotoneaster and six plants each of the other three species, spaced two feet apart.

The area was irrigated on June 5 and the chemicals were applied two days later in 21 gallons of water per acre as an over-all spray at a pressure of 25 psi. Sesone, NPA, monuron, diuron, amitrol, dalapon, CDEC, EPTC, and simazin were used at rates and in combinations indicated in Table 1. The soil was moist at time of treatment and .01 inch of rain fell shortly after the chemicals were applied.

Results.

Weed control and injury ratings were made on July 15. The weeds present were lambsquarters *(Chenopodium album)*, pigweed *(Amaranthus retroflexus)*, purslane *(Portulaca oleracea)*, ragweed *(Ambrosia artemisifolia)*, and crabgrass *(Digitaria sanguinalis)*. Weed counts and weights were obtained on August 26. The weed counts and weights correlated with the ratings made previously. As shown in Table 1, commercially acceptable weed control, without causing injury to any of the species in the test, was obtained by the following: 4 pounds per acre of sesone, 2 and 4 pounds per acre of simazin, and the combination of 4 pounds of NPA and 4 pounds of sesone per acre. The latter treatment was not significantly better than that of sesone alone.

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[1]Journal Article No. 2359 of the Michigan Agricultural Experiment Station.

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Table 1. Weed control and injury ratings of herbicidal treatments applied to four species of ornamentals.

Chemical	Rate lb/A act. ingr.	Ratings	
		Weed control[a] (av. 3 obs.)	Injury[b] (av. 12 obs.)
None	–	1.0	1.0
Sesone	4	6.7	1.3
NPA	4	4.0	1.2
Monuron	1	7.0	2.8
Diuron	1	7.7	4.1
Simazin	2	8.7	1.3
Simazin	4	8.7	1.2
EPTC	6	5.3	1.9
CDEC	4	3.0	1.1
Amitrol	6	7.3	4.8
Diuron + sesone	1 + 4	7.7	4.4
NPA + sesone	4 + 4	8.0	1.5
Dalapon	5	3.7	1.1
Dalapon + diuron	5 + 1	8.3	3.8
LSD 5%		2.0	1.5
LSD 1%		2.9	2.0
Coefficient of variation		21%	22%

[a]Weed control rating scale: 1.0 = no control, 6.0 = commercially acceptable control, 9.0 = eradication of weeds.
[b]Injury rating scale: 1.0 = no injury, 9.0 = complete kill.

All of the four species responded similarly to the chemicals; however, there was a difference in susceptibility to injury by all chemicals. Monuron and diuron, alone or in combination with other chemicals, caused injury to all of the species tested. Amitrol caused severe injury to all species. NPA, EPTC, CDEC, and dalapon gave some weed control, and did not injure any of the species but the weed control was not considered commercially acceptable at the rates used.

Table 2 gives the average injury ratings for all chemicals on the different species. *Syringa chinensis* was the most susceptible, and *Taxus media* 'Hicksi' the most resistant species. There was a distinct difference in susceptibility of the two species of taxus. This indicates the need for testing each species before making a recommendation.

Weed counts and fresh weights of weeds taken on August 26 indicated that the best weed control at this time was from the simazin

Table 2. Average injury ratings from fourteen herbicidal treatments on four species of ornamentals.

	Injury ratings [a,b]
Syringa chinensis	2.64
Taxus cuspidata capitata	2.45
Cotoneaster acutifolia	1.72
Taxus media 'Hicksi'	1.53
LSD 5%	.32
LSD 1%	.42
Coefficient of variation	39%

[a]Rating scale: 1 = no injury; 3 = severe chlorosis; 5 = death of plant.
[b]Av. 45 observations.

treatments. The two and four pound rates of simazin averaged 0.3 and 0.1 ounces of weeds per square foot respectively while the untreated plots, which had been hoed once, averaged 4.6 ounces of weeds per square foot.

Observations made again in October of 1957 and June of 1958 indicated the following: 1) Weed control was still excellent in the simazin plots and there was no injury to any of the species tested; 2) growth was poorest on those plots which had been rated as severely injured by the treatment the previous year. In those plots which had received amitrol, the lilacs and cotoneasters were dead. All of the species in plots receiving diuron or monuron, alone or in combination, exhibited signs of injury in the spring of 1958.

A further comparison of simazin, sesone, and neburon was made on this planting in June, 1958, by applying the three materials in plots running perpendicular to the planting and across the first treatments of the experiment. The materials were used at four pounds per acre of active material with each plot, 44 by 4 feet, on each of the four species and allowing a check plot for each species. One-half inch of water was applied by overhead irrigation immediately after the chemicals were applied.

The early weed control was excellent with all materials, but by August, only the simazin was giving commercial weed control as is shown in Figure 1. None of the materials caused injury to any species

Figure 1. Weed control with four pounds per acre of simazin on *Taxus media* 'Hicksi' (left) and *Cotoneaster acutifolia* (right) applied June 9. Control, center. Photographed August 15, 1958.

and the application of four more pounds of simazin on the previous years' simazin plots had not caused any visible injury.

FALL FUMIGATION TREATMENTS COMPARED WITH SIMAZIN SPRAY TREATMENTS ON ORNAMENTALS

Materials and methods.

In the fall of 1957, a comparison was made of three fumigants with simazin for weed control on several species.

The plots were laid out in a randomized block with three replications. Each plot was 7 by 14 feet. One pound of methyl bromide (MC–2), under a plastic cover, one quart of sodium N–methyl dithiocarbamate dihydrate (Vapam), and three-fourths pound of 3,5–dimethyltetrahydro–1,3–5,2H thiadiazine–2–thione (Mylone) were applied on the 98-square foot plots on September 18. One inch of water was applied with overhead irrigation immediately after the chemicals were applied.

On October 9, fourteen days after application of the soil fumigants, the following plant species were planted at random in each plot: *Euonymus fortunei vegetus, Juniperus chinensis pfitzeriana, Forsythia intermedia, Pyracantha coccinea,* and *Tulipa* sp. On October 12, the simazin treatment was applied at the rate of four pounds per acre as an over-all spray.

Results.

Observations made on June 12, 1958, indicated that weed control was excellent from all treatments, as shown in Table 3. Weeds present were *Stellaria media, Capsella bursa-pastoris, Rumex acetosa,* and *Trifolium repens.*

Table 3. Weed control ratings in spring from previous fall applications of fumigants and simazin.

Chemical	Rate	Rating[a]
None	—	1.0
Mylone	¾ lb per 100 sq. ft.	7.0
Vapam	1 lb per 100 sq. ft.	6.7
MC-2	1 lb per 100 sq. ft.	7.3
Simazin	4 lb per acre	9.0
LSD 1%		2.7

[a]Rating scale: 1 = no control; 6 = commercial control; 9 = eradication.

Two other observations of interest were that the simazin completely eradicated all weeds, and the MC–2 was not effective in controlling *Trifolium repens.*

None of the treatments except simazin caused any apparent damage to the ornamentals. Simazin caused severe injury to euonymus forsythia and pyracantha as can be seen in the injury ratings shown in Table 4. These results are in agreement with those of Ticknor and Gasiorkiewicz (11).

Table 4. Injury ratings in spring on various species from fall application of four pounds per acre of simazin.

Species	Rating[a]
Euonymus fortunei vegetus	8.7
Juniperus chinensis pfitzeriana	1.0
Forsythia intermedia	4.7
Pyracantha coccinea	6.0
Tulipa sp.	1.0
LSD 1%	2.9

[a]Rating scale: 1 = no injury; 9 = dead plants.

GREENHOUSE TESTS

In field tests during 1957, simazin for control of annual weeds and dalapon for control of quackgrass had given outstanding results. Therefore, further tests of these materials in the greenhouse on more species, and evaluation of simazin and other triazine compounds seemed desirable. In addition, tests were also run to determine the response of different species of ornamentals to various rates of simazin when applied on different soil types and on plants held at different temperatures.

Materials and methods.

In a study to compare simazin, other triazine compounds, and rates of dalapon, the species used were: *Juniperus chinensis pfitzeriana, Taxus cuspidata 'Expansa', Hedera helix baltica, Pachysandra terminalis,* and *Euonymus fortunei vegetus.* The rates and chemicals used are in Table 5.

Table 5. Injury from herbicides applied in the greenhouse on five species of ornamentals.

Chemical	Rate lb/A	Injury rating[a]					
		Juniperus chinensis pfitzeriana	*Taxus cuspidata* 'Expansa'	*Hedera helix baltica*	*Pachysandra terminalis*	*Euonymus fortunei vegetus*	Average for treatments
None	—	2.0[b]	1.0	1.0	1.2	1.0	1.24
Simazin	4	1.0	1.2	1.2	2.0	1.6	1.40
2-chloro-4-diethylamino-6-isopropylamino-*s*-triazine (G30031)	4	1.0	1.0	2.0	1.6	3.4	1.80
2-chloro-4-ethylamino-6-diethylamino-*s*-triazine (G27901)	4	1.2	1.6	1.0	1.4	1.2	1.28
Dalapon	10	1.6	1.8	1.4	2.8	1.4	1.80
Dalapon	20	1.8	2.6	1.8	3.2	1.0	2.10
Simazin + dalapon	4 + 10	1.8	2.4	3.6	3.4	2.4	2.72
Average for species		1.49	1.66	1.71	2.22	1.72	
LSD 5%		Between species averages .34			Between treatment averages		.39
LSD 1%				.44			.52

LSD interaction (difference of a difference) of treatments and species equals 1.2 at 5% level, 1.6 at 1% level.

[a]Rating scale: 1.0 = no injury; 3.0 = severe chlorosis; 5.0 = death of plant.
[b]"Injury" ratings on checks in this and later tables reflect the fact that all plots were rated without looking at labels, and that these actually looked this way. Such ratings reflect plot and rating variability.

Individual plants were placed in four inch fibre pots during the week of November 18. Treatments were applied on November 25 with a small sprayer using carbon dioxide as a source of pressure. Each treatment was replicated five times and all treatments and species were completely randomized in a greenhouse where a 70° F. night temperature was maintained. The day temperature fluctuated between 70°–85° F.

In the second test, the following factors were included: Species *(Juniperus chinensis pfitzeriana, Euonymus fortunei vegetus, Hedera helix baltica,* and *Hemerocallis);* rate of simazin (two, four, and eight

pounds per acre); soil type (sandy loam, clay loam, and muck); and night temperatures (60° and 70° F.). All possible combinations of these factors were used with four replications of each. The treatments were completely randomized with all species under each temperature condition.

Weed seeds were sown in each pot, but weed growth was too poor to use as an index of weed control. Injury ratings were made on May 14.

Results.

The injury caused by the various herbicides is indicated in Table 5. Neither simazin nor the other triazine compounds applied alone caused injury to any species except euonymus, which was sensitive to Propazine. Pachysandra and taxus seemed to be particularly sensitive to dalapon. The combination of simazin with dalapon injured all species except juniper and, in general, was more toxic than either chemical alone. The evidence in this test indicates that there may have been a synergistic effect from these two herbicides which was responsible for the severe injury on certain species.

An analysis of variance was run for these factorial experiments, which included species, soil type, rate, and temperature. At the low temperature of 60° F., only the baltic ivy showed any injury symptoms from applications of simazin, as shown in Table 6. The lack of response may have been due to lack of growth at the lower temperature.

Table 6. Response of the different species to the four treatments and on the three soils at the two temperatures studied.[a]

Night temperature	Injury rating[b]			
	Juniperus chinensis pfitzeriana	*Hedera helix baltica*	*Euonymus fortuni vegetus*	*Hemerocallis*
60°	1.0	1.5	1.0	1.0
70°	1.4	1.6	1.9	1.2

[a]F value for interaction significant at 5% level.
[b]Rating scale: 1.0 = no injury; 3.0 = severe chlorosis; 5.0 = death.

The data in Table 7 indicate that as the rate increased, injury from simazin increased with the exception of hemerocallis. Both baltic ivy and euonymus were more sensitive to simazin than either Pfitzer's juniper or hemerocallis.

With the exception of Pfitzer's juniper, more injury occurred on the species on sandy loam than on either the clay loam or muck soil, as may be seen in Table 8. At the 70° night temperature, all plants of the baltic ivy and euonymus treated with eight pounds of simazin on the sandy soil were killed or had severe chlorosis.

Table 7. Effect of rate of simazin on injury to four species on the three soil types and at two temperatures.[a]

Rate simazin lb/A	Injury ratings[b]				
	Juniperus chinensis pfitzeriana	*Hedera helix baltica*	*Euonymus fortunei vegetus*	*Hemerocallis*	Average for rate
None	1.0	1.1	1.1	1.0	1.1[c]
2	1.0	1.2	1.4	1.1	1.2
4	1.2	1.5	1.4	1.2	1.3
8	1.4	2.4	1.8	1.1	1.7
LSD at 5%					0.5
LSD at 1%					0.6

[a]F value for interaction significant at 5% level.
[b]Rating scale: 1 = no injury; 3 = severe chlorosis; 5 = death of plant.
[c]See Table 5[b].

Table 8. The response of four species of ornamentals to four treatments on various soils.[a]

Soil type	Injury rating[b]				
	Juniperus chinensis pfitzeriana	*Hedera helix baltica*	*Euonymus fortunei vegetus*	*Hemerocallis*	Average for all varieties
Muck	1.4	1.1	1.2	1.0	1.2
Clay loam	1.1	1.3	1.5	1.0	1.2
Sandy loam	1.0	2.3	1.6	1.3	1.6
LSD at 1%					0.4

[a]F value for interaction significant at 1% level.
[b]Rating scale: 1.0 = no injury; 5.0 = plants dead.

GROWER SURVEY

In order to compare recommended herbicides with simazin it was decided that it would be advisable to enlist the aid of growers of nursery stock.

Materials and methods.

In May of 1958, the following materials were sent to fifty Michigan nurserymen: 1) sesone, 2) neburon, 3) simazin, and 4) a check, which (unknown to the grower) was DDT.

Each material was weighed out and identified only by a code letter. There was just enough material, when applied to an area of 400 square feet, to be equivalent to four pounds per acre for the sesone and neburon, and two pounds per acre for the simazin and the check (DDT). Special directions were provided for the application of the material and a warning was made to apply the materials only to woody ornamentals.

A questionnaire was sent with the directions, and a follow-up letter was mailed in August. Each grower was asked to rate the effectiveness of the materials after two, four, and six weeks, and to list the injury, if any, as well as the species treated. Of the fifty growers asked to cooperate, eight fulfilled all the suggestions and three other reports were not complete enough for use in this analysis.

Results.

None of the materials tested were reported by the cooperators to have caused injury to any species. A summary of the weed control ratings is presented in Table 9. The best material was simazin, followed by neburon and sesone. Of the eight growers reporting, seven rated simazin as superior and one equal to neburon and sesone.

The weed control for the 2, 4, and 6 week periods decreased with time for neburon and sesone, but did not change for simazin, as is shown in Table 9. Figure 2 shows a portion of one of the tests put out by a nurseryman.

Table 9. Summary of weed control ratings from all treatments at eight nurseries.

Chemical	Rate lb/A	Weed control ratings[b]			
		Weeks after application[a]			Average
		2	4	6	
Check (DDT)	2	1.6	1.2	1.1	1.3[c]
Sesone	4	3.2	2.9	2.4	2.8
Neburon	4	3.9	3.0	2.5	3.1
Simazin	2	4.5	4.5	4.4	4.5
LSD at 1%					0.8

[a]F value for interaction of 3 chemicals and time significant at 5% level.
[b]Rating scale: 1.0 = no weed control; 3.0 = commercial weed control; 5.0 = eradication.
[c]See Table 5[b].

Figure 2. A test applied by a nurseryman to lining-out-stock. Four pounds of simazin per acre in background, check in foreground. Plant species from left to right are: *Juniperus chinensis pfitzeriana, Taxus media* 'Hicksi', and *Juniperus chinensis pfitzeriana.*

SUMMARY

From this study, it may be concluded that sesone, neburon, dalapon, and simazin will effectively control weeds in nurseries without causing severe injury to many species. Of these materials, simazin

appears to be the most promising because of its excellent weed killing properties, long residual action, and the number of species which are tolerant. A list of these species is presented in Table 10.

Table 10. Tolerance of ornamentals to simazin.

Resistant species
Acer saccharum (1)[a]
Acer platanoides (1)
Berberis sp. (2)
Cotoneaster acutifolia (3)
Crataegus oxyacantha (1)
Gleditsia triacanthos (2)
Hedera helix baltica (2)
Hemerocallis fulva (1)
Hibiscus syriacus (1)
[b]Juniperus chinensis pfitzeriana (7)
[b]Juniperus communis (2)
[b]Juniperus virginiana glauca (1)
Malus sp. (2)
[b]Pachysandra terminalis (3)
Paeonia officinalis (1)
[b]Picea glauca (2)
[b]Picea pungens (2)
[b]Pieris japonica (1)
[b]Pinus nigra (1)
[b]Pinus sylvestris (2)
[b]Pinus thunbergi (1)
[b]Platanus occidentalis (1)
Rosa, H.T. (1)
Syringa chinensis (1)
Taxus sp. (10)
[b]Thuja occidentalis (2)
Tulipa sp. (1)
[b]Ulmus americana (1)
Viburnum carlesi (1)
Vinca minor (1)
Susceptible species (4 lb/A)
[c]Buxus sempervirens (1)
Euonymus fortunei vegetus (4)
Forsythia intermedia (3)
[c]Fraxinus pennsylvanica lanceolata (1)
[c]Ilex crenata convexa (1)
[c]Juniperus horizontalis plumosa (1)
Pyracantha coccinea lalandi (3)

[a]Numbers in parentheses indicate number of observations.
[b]Plants observed only at 2 lb/A, all others at 4 lb/A.
[c]Plants were not injured at 2 lb/A.

All of these species have been included in one or more replicated trials made by the authors. There are undoubtedly errors in the list because of limited data, but it is believed that this type of list will be valuable to the grower of nursery stock.

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Mode and Rate of Release of Isopropyl *N*–(3–Chlorophenyl)Carbamate from Several Granular Carriers

L. L. Danielson[1]

The commercial use of herbicides formulated on granular carriers has expanded rapidly and in many instances has preceded research on their development and properties. Experimental studies have been concerned primarily with field problems involving the control of weeds in a particular weed-crop situation. Conflicting results obtained in both the experimental and the practical use of granular herbicides have emphasized the need for basic information on the mode of action of granular-herbicide formulations and the effect of environment and cultural techniques on their use.

The experiments described herein were conducted to measure plant responses to isopropyl *N*–(3–chlorophenyl)carbamate (CIPC) formulated on several granular carriers. Previous research (1, 2, 6, 9) indicated the importance of both the contact activity and the vapor activity of this chemical. The present investigation included a study of these aspects of its activity.

Bioassays have been used successfully by a number of workers to study the quantitative aspects of herbicidal activity. Swanson et al. (9) used a bioassay method involving the measurement of the rate of elongation of the primary root of germinating cotton seed to determine the contact and vapor activity of CIPC in petri dishes. Ready and Grant (5) used a similar method involving the measurement of the rate of elongation of the primary root and hypocotyl of germinating cucumber seed to analyze for 2,4–dichlorophenoxyacetic acid (2,4–D). Mitchell et al. (4), Swanson (8), and Thompson (10), have described the successful use of bioassays to measure the growth-activity of chemicals.

Initial experiments were conducted to select a responsive, acceptable test plant for a bioassay method for CIPC. The elongation of the primary roots and shoots of buckwheat, corn, cotton, and cucumber seedlings was measured in contact with CIPC and exposed to its vapors. All the test species used were responsive to CIPC, but they varied in sensitivity and in the time required to show response. Cucumber was selected as the most satisfactory test plant. Accordingly, a bioassay method of analysis for CIPC by the use of germinating cucumber seed was adopted as a standard procedure for use in the granular-herbicide evaluations.

Materials and Methods

National Pickling cucumber was the standard variety used. Covered petri dishes, 100 x 15 mm, were fitted with Whatman No. 2 filter papers, and 7 ml of distilled water were added to each dish. Contact studies with crystalline CIPC were conducted by pipetting 1 ml of an acetone solution of CIPC on the surface of the water

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in the petri dish and allowing sufficient time for the acetone to evaporate before placing 10 cucumber seed in the dish and covering it. The acetone solutions were prepared to contain the desired amount of CIPC in 1 ml of acetone for each dish.

In the contact studies, granular formulations of CIPC were placed on the dry filter paper before the addition of water. After addition of the water, the granules were distributed over the surface of the filter paper by giving the dish a momentary swirling motion. The seed were then placed in contact with the granules.

The vapor activity of crystalline CIPC and granular formulations was studied by placing the acetone solution or granular test material in an aluminum foil container in the petri dish as shown in Figure 1. The acetone was evaporated before placing the seed in the dish and closing it.

The granular formulations were prepared so that all carriers contained the same percentage of CIPC by weight. Acetone solutions of CIPC were used to treat the granules. In each instance, 1 gm of the granular formulation was used per dish.

Dry and water-moistened granules were used in the vapor studies to give information on the effect of very low and very high moisture conditions on the activity of granular formulations. In these studies, a measured amount of water was used to moisten the granules in the aluminum foil container, and care was taken so that water did not stand in the container.

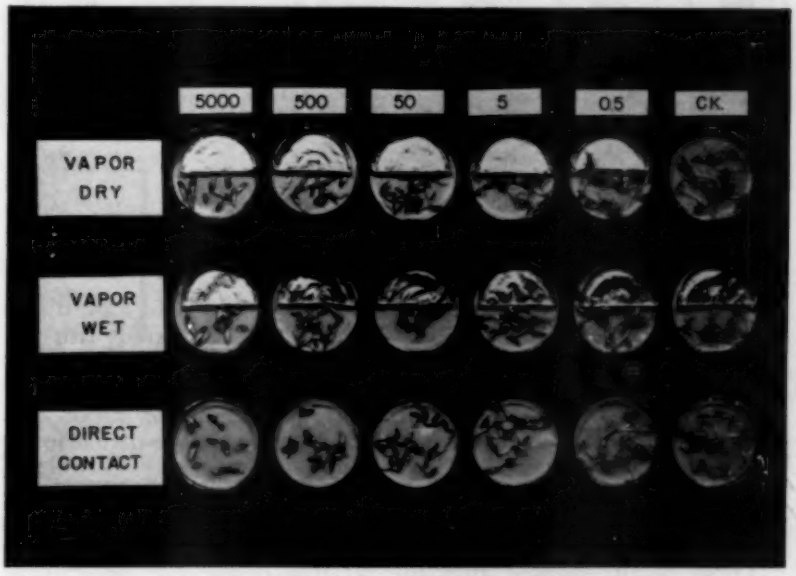

Figure 1. Petri-dish assemblies used in the bioassay with germinating cucumber seed. Note semi-circular aluminum foil containers in the top two rows. Vapor activities and contact activities of 0.5 to 5000 gammas of CIPC crystals per dish are compared.

The covered petri dishes were placed in the dark for 96 hours at 28° C to allow germination of the cucumber seed. Hypocotyl and primary root lengths were measured to the nearest millimeter at the end of this period. When the measurements could not be made immediately, the seedlings were stored successfully at approximately 4° C for as long as 7 days.

The growth responses were evaluated by expressing the measurements of the treated seedlings as percentages of those of the control series. Distilled-water and carrier controls were included in each germination series. The carrier controls were used as standards for comparison.

RESULTS

Measurements of the rates of elongation of the hypocotyl and the primary root of cucumber seedlings were found equally effective for determining the vapor and contact activities of CIPC, Table 1. Hypocotyl length is measured conveniently with a millimeter rule and such measurement was adopted as the standard in these studies.

Table 1. Effects of vapor and contact activities of CIPC crystals on elongation of the primary root and the hypocotyl of cucumber seedlings.

Amount of herbicide per dish (gammas)	Method of exposure	Hypocotyl		Primary root	
		Mean length (mm)	Relative length as percent of control	Mean length (mm)	Relative length as percent of control
0	Contact	39	100	72	100
	Vapor	27	100	56	100
0.5	Contact	34	87	60	83
	Vapor	28	104	53	95
5	Contact	19	49	28	39
	Vapor	13	48	21	38
50	Contact	8	21	11	15
	Vapor	8	30	12	21
500	Contact	5	13	6	8
	Vapor	6	22	9	16
5000	Contact	3	8	2	3
	Vapor	4	15	4	7

A seed-germination series, Figure 1, shows the effect of direct contact of the seedlings with crystalline CIPC compared with the effect of exposure to the vapors of the dry and moistened crystals of the chemical. The responses to the vapors and to contact with the chemical were similar in magnitude, Figure 2. Major vapor and contact responses occurred in the concentration range of 0.5 to 50 gammas of CIPC per dish.

Water-moistening the uncalcined attapulgite granules treated with CIPC increased their vapor activity as illustrated in the germination series in Figure 3 and shown graphically in Figure 2.

These results showed that the relative herbicidal (or growth-inhibitory) activity of uncalcined attapulgite granules treated with CIPC could be successfully evaluated by this method. Accordingly, the method was used to evaluate CIPC on uncalcined attapulgite

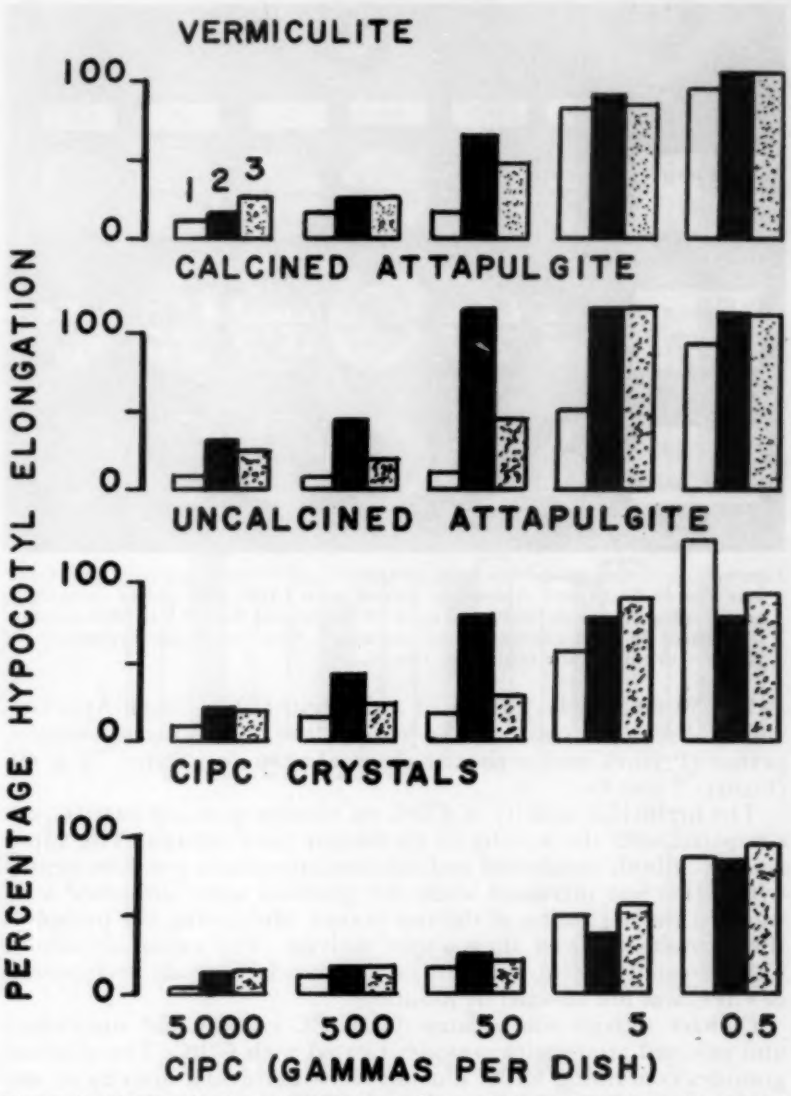

Figure 2. Hypocotyl elongation of cucumber seedlings exposed to (1) contact action, (2) dry-vapor action, and (3) wet-vapor action of crystalline CIPC, and CIPC-treated vermiculite, calcined attapulgite, and uncalcined attapulgite. The percentages are of the hypocotyl elongation of untreated seedlings.

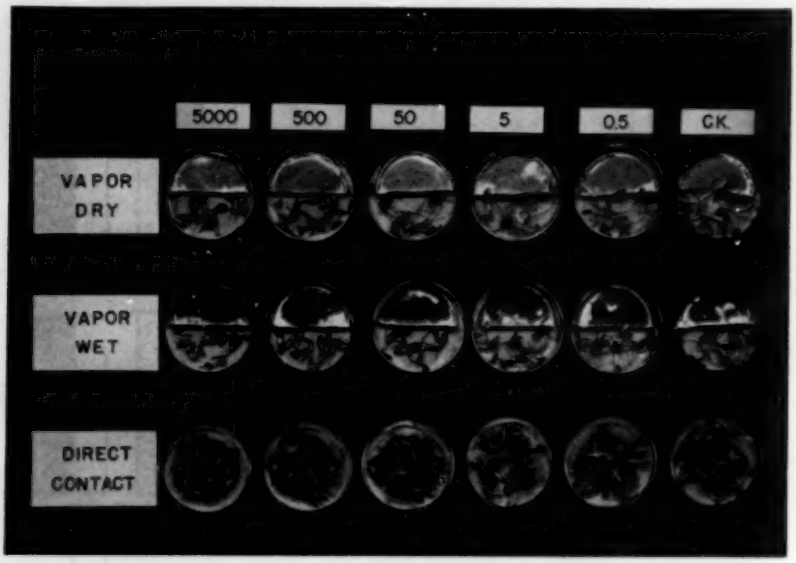

Figure 3. Petri-dish assemblies in a comparison of contact activities and vapor activities of uncalcined attapulgite treated with CIPC. One gm of attapulgite granules having the desired amount of CIPC ranging from 0.5 to 5000 gammas was placed in each aluminum foil container. Note the moist appearance of granules in the middle horizontal row.

(RVM 30–60 Attaclay), calcined attapulgite (LVM 30–60 Attaclay), vermiculite (Vermiculite No. 3), pyrophyllite (Pyrax coarse granules), perlite (Perlite), and activated charcoal granules (Darco 20 x 40) (Figures 2 and 4).

The herbicidal activity of CIPC on various granular carriers was compared with the activity of chemically pure crystals. The vapor activity of both uncalcined and calcined attapulgite granules treated with CIPC was increased when the granules were moistened with water at the beginning of the test period. Moistening the pyrophyllite granules reduced their vapor activity. The vapor activity of CIPC treated perlite, vermiculite, activated charcoal, and crystals of CIPC was not affected by moisture.

Contact activity was greatest for CIPC crystals and uncalcined and calcined attapulgite granules treated with CIPC. The charcoal granules containing CIPC did not show herbicidal activity at any of the concentration levels evaluated. CIPC-treated perlite, vermiculite, and pyrophyllite granules were intermediate in contact herbicidal activity.

DISCUSSION

The method of bioassay for contact and vapor activities of CIPC described herein requires a minimum of equipment, time, and experience for effective use. The concentration range of 0.5 to 5000 gammas of CIPC was chosen on the basis of preliminary experiments.

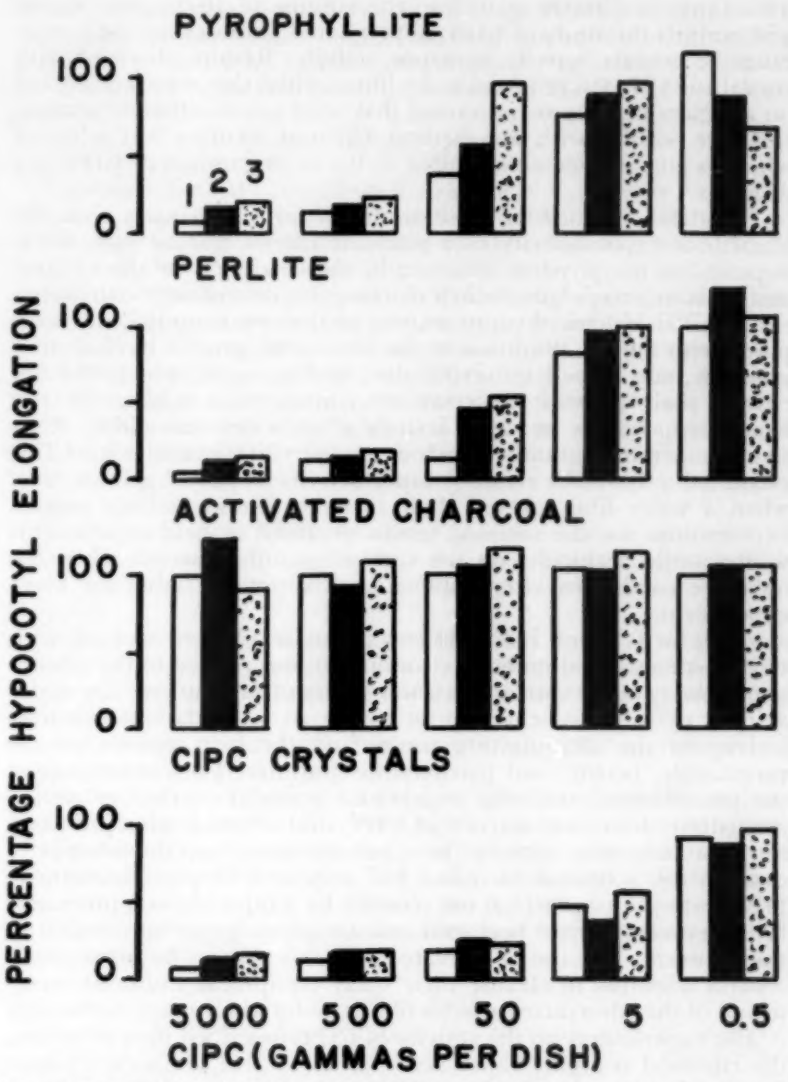

Figure 4. Hypocotyl elongation of cucumber seedlings exposed to (1) contact action, (2) dry-vapor action, and (3) wet-vapor action of crystalline CIPC, and CIPC-treated pyrophyllite, perlite, and activated charcoal. The percentages are of the hypocotyl elongation of untreated seedlings.

This range is directly related to the volume of 10-cm petri dishes and permits the study of CIPC-impregnated granules having a wide range of contact activity or vapor activity. Results obtained with crystalline CIPC were found to be linear when they were considered on a logarithmic basis indicating that semi-quantitative determinations are possible with this method. Greatest accuracy was achieved when specific studies were limited to 0.5 to 50 gammas of CIPC per dish.

The data obtained in these studies show very clearly that the immediate vapor activity of a granular carrier treated with CIPC depends on the physical structure of the carrier. Granules of such materials as attapulgite, which disintegrate on contact with water, exhibited an enhanced vapor activity in the moist condition as compared with the dry condition at the 50 to 5000 gamma level. Porous granules such as perlite, vermiculite, and charcoal, which did not change their physical structure on contact with water, did not change appreciably in vapor activity when water was added. Relatively impervious granules, such as pyrophyllite, coated with CIPC exhibited a markedly reduced vapor activity at the 50 gamma level when a water film covered their surfaces. These findings suggest explanations for the variable results obtained in field experiments with volatile herbicides on the various granular carriers when the moisture conditions after application are not the same for every experiment.

Vapor activity of the different granular carriers treated with CIPC, when studied under dry conditions, was related to the adsorptive capacity of the carrier. This was evident from the very low vapor activity of activated charcoal as compared with the intermediate activity of the attapulgite granules and the high activity of the vermiculite, perlite, and pyrophyllite granules. These data suggest the possibility of selecting impervious granular carriers to obtain immediate short-term activity of CIPC and of more adsorptive carriers for long-term activity. In a related situation, the adsorptive capacity of activated charcoal for isopropyl–*N*–phenylcarbamate [IPC] was put to practical use recently by Ripper (7) as a protective layer between planted beet seed and a pre-emergence application of the chemical. The use of activated charcoal slurries by many weed-control scientists to cleanse their spray equipment indicates recognition of the adsorptive capacity of this material for many herbicides.

The experiments on the activity of CIPC described here show that the chemical is highly vapor active at 28° C, the temperature used for germination of cucumber seed in these bioassays. Information is needed on the effect of a wide range of temperatures on the rate of evaporation of CIPC from the crystalline form and from granular carriers treated with the chemical. Data on the effect of light on various formulations of this chemical will also be necessary to explain its activity.

Techniques of formulation of CIPC on granular carriers must be studied to determine the extent to which the materials and methods of formulation can be used to hasten or delay action of the chemical

or to provide other desired effects on crop plants and weeds. An interesting recent evaluation of bioassay methods for measuring herbicide volatility by Leasure (3) indicated very clearly the need for a study of materials used in formulation as they affect vapor activity.

SUMMARY

A bioassay for CIPC based on the rate of elongation of the hypocotyls of germinating cucumber seed in petri dishes was used to determine the activity of the chemical. The bioassay was used to measure the response of cucumber seedlings to vapor and contact activity of crystalline CIPC and CIPC formulated on granular carriers. The pronounced contact and vapor activities of CIPC crystals were used as the standards for comparison. CIPC at the rate of 0.5, 5, 50, 500, or 5000 gammas to each 100-mm x 15-mm petri dish was used to establish a constant chemical-to-air-volume relation for the assay. These amounts of the chemical were introduced on 1 gm of each of the granular carriers studied. An aluminum foil container was placed in each petri dish to hold the test herbicide in the vapor studies.

Contact activity of CIPC from the granular carriers, in order of activity beginning with the highest, was: calcined attapulgite, uncalcined attapulgite, pyrophyllite, perlite, vermiculite, and activated charcoal.

Vapor activity of CIPC from the formulated granular carriers was related to their physical structure and adsorptive capacity. Vapor action of treated attapulgite granules was enhanced by changing their physical structure by moistening with water. Carriers which did not change physical structure on contact with water displayed unchanged or reduced vapor activity.

Relatively impervious carriers of low adsorptive capacity such as pyrophyllite and vermiculite were highly contact- and vapor-active. Mildly adsorptive clays such as the attapulgites were intermediate in vapor and contact activities. The highly adsorptive activated charcoal granules held CIPC tenaciously and neither vapor nor contact activity was observed at the standard levels of concentration used in these experiments.

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Susceptibility of Velvet Mesquite to an Amine and an Ester of 2,4,5–T as Related to Various Biological and Meteorological Factors[1]

FRED H. TSCHIRLEY and HERBERT M. HULL[2]

HERBICIDAL applications to mesquite in Arizona (17), New Mexico (21), and Texas (5) indicate that the low-volatile esters of 2,4,5–trichlorophenoxyacetic acid (2,4,5–T) are usually more effective than amine salt formulations, but there have been instances where the reverse was true. This inconsistency has occurred only in isolated cases, but was of interest in that factors which affect differential susceptibility to the ester and the amine formulations are important and should be more fully understood. The purpose of this study was to determine whether mesquite has a different period of susceptibility to the ester than to the amine. Various biological and meteorological factors were also determined to test their relation to mesquite susceptibility.

An added impetus to this study was provided by recent research (7) which showed that the amine was consistently better than the ester when tested at relatively high concentrations on greenhouse-grown seedlings. The greater effectiveness of the amine was difficult to explain in view of the mechanics of absorption as discussed by Crafts (2) because it was assumed that cuticular development was similar in the case of greenhouse- and field-grown seedlings. Subsequent anatomical work at this laboratory (8) demonstrated that greenhouse-grown mesquite develops little or no cuticle. This fact provides at least a partial explanation for the difference in results obtained with the ester and the amine in greenhouse and field.

The occasional superiority of the amine in field tests, work by Hoshaw[3] that showed a gradual increase of cuticle thickness as the season progressed, and work by Kurtz (10) showing that wax esters and acids increase with age in a variety of desert plants suggested that the amine might be more effective than the ester if applied early in the season prior to appreciable cuticle development.

Detailed carbohydrate analyses of the mesquite tree[4] and the dependence of 2,4–dichlorophenoxyacetic acid translocation on simultaneous movement of carbohydrates (6, 9, 11, 13, 18, 19) suggested the desirability of studying seasonal variation in carbohydrate content as related to herbicidal sensitivity. The root xylem was chosen for detailed study in the present investigation because pre-

[1]Cooperative contribution of the Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, and the Rocky Mountain Forest and Range Experiment Station, Tucson, Arizona, respectively.

[2]Range Conservationist and Plant Physiologist, respectively, Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, Box 5735, Tucson, Arizona.

[3]Hoshaw, Robert W. Studies on the anatomy of mesquite leaves. 19 pp. Bot. Dept., Univ. of Arizona, 1955 (Cooperative study between the Ariz. Agr. Expt. Sta. and the U. S. Forest Service.)

[4]Hull, Herbert M. Unpublished data.

vious analyses indicated that it was one of the major storage organs for carbohydrates, and showed relatively wide seasonal variation in certain carbohydrates.

There is no quantitative measure of the period when mesquite is most susceptible to foliar applications of 2,4,5–T. Stage of growth has been used as a criterion of susceptibility, but such observations are of necessity subjective and therefore subject to variation depending upon the person making the observation. In the hope of finding a quantitative measure of susceptibility, air temperature, soil temperature, relative humidity, leaf moisture, and the nature and content of carbohydrates in the root xylem at the time of treatment were considered as possible factors which might influence the herbicidal susceptibility of mesquite.

MATERIALS AND METHODS

The design for the field applications in this study was a randomized block with two replications. Twenty velvet mesquite *(Prosopis juliflora* var. *velutina* (Woot.) Sarg.) trees were treated in each of two replications on each spray date starting April 29 and ending July 1, 1955. Two formulations of 2,4,5–T,[5] a propyleneglycolbutylether ester and a triethylamine salt, were used at a concentration of 4.0 lb ahg. The carrier for the ester was a 1:4 emulsion of diesel oil and water, and the carrier for the amine was a 1:4 emulsion of nontoxic oil (S/V Sovaspray 100, now called Mobilsol 100)[5] and water. Applications were made with a compressed-air spray can at 30 lb pressure using an 8001 Teejet tip. Applications were light but covered the plant thoroughly in an attempt to simulate an aerial spray as closely as possible. The ester contained a surfactant in the commercial formulation, but the amine did not. No surfactant was added to either of the formulations. The emulsifying property of the amine formulation itself was sufficient to hold a good emulsion with occasional agitation.

Growth and development of the trees to be sprayed were obtained by observing and tabulating the onset and development of leaves and their relative maturity, onset and development of flowers, and the onset and development of fruits. Leaf samples were taken from six of the twenty trees on each treatment date. These samples were weighed, oven-dried to constant weight at 70° C, and re-weighed for calculation of leaf moisture. Leaf moisture was expressed as percentage of fresh weight.

Air temperature, relative humidity and soil temperature at a 25-cm depth were taken at approximately 9 a.m. on each spray date.

Samples of the root xylem were taken from just below the root crown for carbohydrate analyses from twelve of the twenty treated trees on each spray date. A 1.5- x 5-cm section was cut out with a wood chisel. The block of tissue separated easily at the cambium

[5]The authors wish to acknowledge the cooperation of the Dow Chemical Company in supplying the herbicides and that of the Socony Mobil Oil Company, Inc., in supplying the nontoxic oil used in this study.

layer. Three mm of the xylem adjacent to the cambium was excised and placed under refrigeration for transport to the laboratory. The samples were dried immediately at 80° C for several hours in a ventilated oven and further dried at 70° C to constant weight after which they were ground to pass a 40-mesh screen. The 12 samples were divided into four groups of three samples each, and extracted with 80 percent ethanol. Total sugars were determined photometrically (14, 20); individual sugars were determined by paper partition chromatography by the ascending technique with Whatman No. 1 filter paper. The irrigating solvent consisted of a mixture of 4 parts *n*-butanol, 1 part glacial acetic acid, and 5 parts water (by volume). Spots were developed on duplicate chromatograms with α–napthol in 95 percent ethanol plus 10 percent (v/v) of phosphoric acid (1) and also by aniline hydrogen phthalate (15). Total starch was determined spectrophotometrically after solubilization with perchloric acid (12, 16). Transmission of the color complex was read at 660 mμ.

The final data on response of mesquite trees to treatment were taken at the end of the third growing season after treatment. Percentage defoliation was estimated for each tree on a scale of one to ten. Plant kill was also tabulated, but the analyses and results are given in terms of defoliation because plant kill was low on all plots and did not vary enough to be significant.

Results and Discussion

Effect of formulation and time of treatment.

An analysis of variance showed that differences between herbicidal formulations and dates of treatment were significant at the 5 percent level of probability. The ester was consistently better than the amine salt as shown in Table 1. In addition, the period of maximum susceptibility was longer with the ester than with the amine salt. In this trial the most effective dates of treatment with the ester were May

Table 1. Mean defoliation of mesquite on each of 12 dates using an ester and an amine salt of 2,4,5–T.

Date of treatment (1955)	Ester		Amine	
	Percentage defoliation	Statistical significance[a]	Percentage defoliation	Statistical significance[a]
Apr. 29	60	e	49	b c d e
May 6	55	e	46	c d e f g h
13	87	a	63	a b
20	71	b c d	47	b c d e f g
26	85	a b	69	a
30	82	a b c	62	a b c
June 3	81	a b c	52	b c d
7	67	c d e	48	b c d e f
10	69	b c d e	41	d e f g h i
16	80	a b c d	38	d e f g h i
24	55	e	31	g h i
July 1	77	a b c d	29	i
Mean	72		48	

[a]Any two means having one or more subscript letters in common are not significantly different. Any two means not having subscript letters in common are significantly different at the 5 percent level. Duncan's multiple range test (3).

13, 26, 30, June 3, 16, and July 1. With the amine, only May 13, 26, and 30 were significantly better than other dates.

The ester was not only more effective on all dates, but the difference in defoliation for the two formulations increased as the seasons progressed. This is shown graphically in Figure 1. It was thought

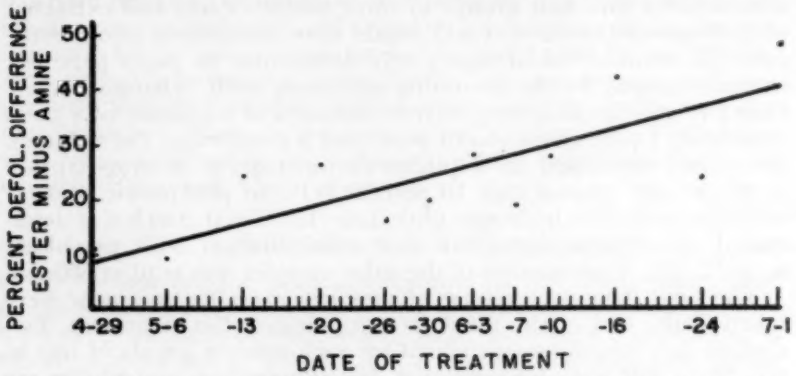

Figure 1. Calculated regression of difference between ester and amine formulations of 2,4,5–T in mesquite defoliation as a function of treatment dates.

when the study was started that the amine would be most effective during the early part of the season before the cuticle had developed to its maximum thickness. Such was the case. Effectiveness of the amine dropped steadily after May 26. This suggests that absorption rather than translocation is the critical factor involved in using the amine for brush control. Recent work suggests that had a surfactant been present in the amine, its effect during the latter part of the season may have been enhanced.

Relation of phenological development to susceptibility.

Stage of growth has been used for a number of years as a criterion for determining the period of maximum susceptibility of mesquite. Specifically, the period at which mesquite can be treated most effectively with a foliage spray is defined as that time when the leaves have reached full size but are still succulent, flower development is complete, and pods have formed but are less than 1 inch long. That definition of susceptibility is adequate in most years, but it has definite limitations. The most serious limitation is the subjective method used to determine the difference between leaves that are full size, but succulent (without a thick, waxy cuticle), and mature leaves (with a thick, waxy cuticle). The differentiation is made by visual and tactile sensations. Leaves not yet mature, though full size, are green and have a soft, velvety feel. As leaves mature they become grey-green, shiny, and hard and slick to the touch. Another limitation is variation in development from tree to tree. There is considerable overlapping between the various growth stages, as shown in Figure 2. A third limitation is that pods sometimes do not start development until after the leaves have a well-developed cuticle.

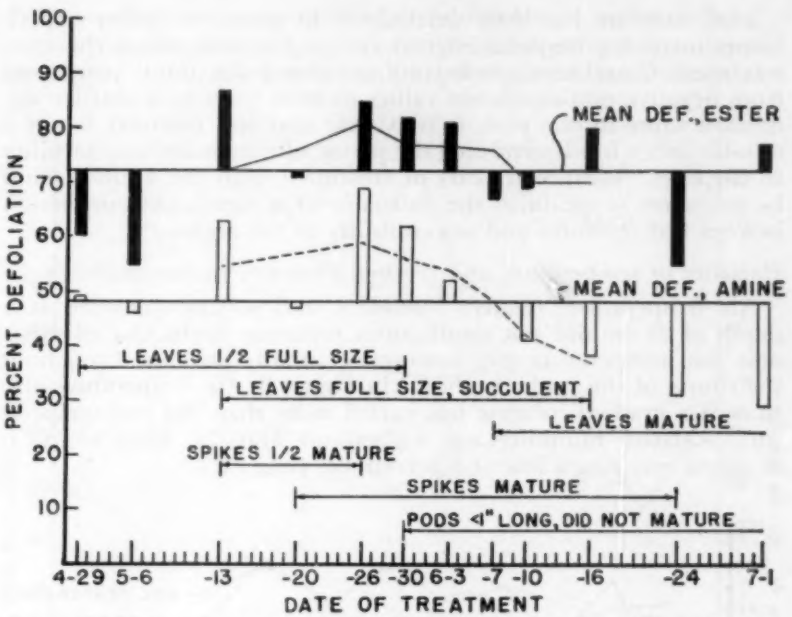

Figure 2. Relation of growth stage to herbicidal susceptibility of mesquite. The bar graph represents defoliation on each date of treatment. The line graph represents an average of 5 consecutive dates of treatment.

Experience with stage of growth as a criterion of susceptibility suggests that leaf maturity is of principal importance. Flowering and fruiting are important only in that they coincidentally occur when mesquite is most susceptible.

The phenological development depicted in Figure 2 is only relative. The date at which a given stage of growth is shown as having started or ended is the date when 50 percent or more of the trees checked exhibited that characteristic. For example, mature leaves are shown for the first time on June 7, but nearly 50 percent of the trees already had mature leaves on June 3. Despite its limitations, stage of growth is still the best method for determining the period of maximum susceptibility to foliage sprays.

Relation of leaf moisture to susceptibility.

The percentage of leaf moisture decreased gradually from a high of 66 percent at the first treatment date to a low of 46 percent at the last treatment date. There were minor fluctuations during the period, but they were not significant. Leaf moisture could not be correlated with herbicidal effectiveness. It is of interest however, that the correlation coefficient was much closer to being significant with the amine than with the ester. Correlation coefficients of 0.517 and —0.027 were obtained for the amine and the ester, respectively. The significant value of r at 5 percent was 0.533 for both formulations.

Leaf moisture has been determined in numerous other experiments involving frequent-interval sprays, but only when the ester was used. Correlation coefficients calculated for those years vary from negative non-significant values in most years to a positive significant value in one year. It is evident that leaf moisture is not a reliable index for determining the period of maximum susceptibility to the ester. Additional years of treatment with the amine would be necessary to establish the existence of a significant correlation beween leaf moisture and susceptibility to the amine.

Relation of temperature and relative humidity to susceptibility.

Air temperature, relative humidity, and soil temperature at a depth of 25 cm did not significantly influence herbicidal effectiveness. Soil temperature did, however, gradually increase throughout the course of the study, as shown in Figure 3. Air temperature also showed a gradual increase but varied more than did soil temperature. Relative humidity was highest on May 26, after which it dropped, reaching a low of 8 percent on June 24.

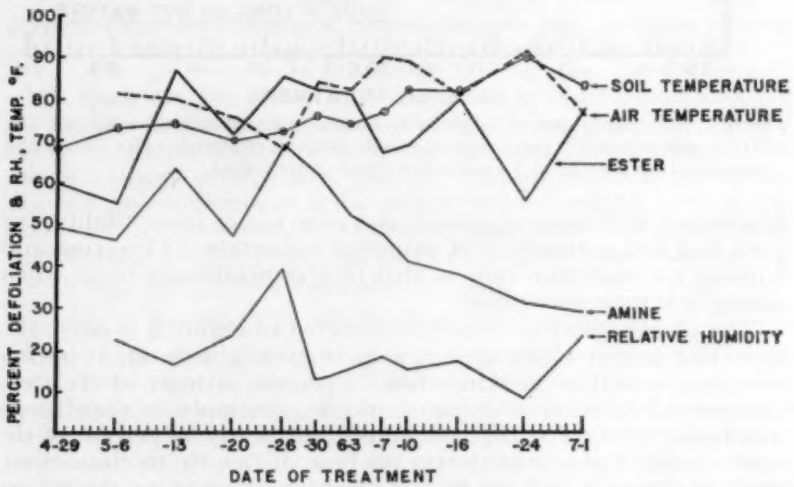

Figure 3. Air temperature, soil temperature, relative humidity and percentage defoliation as a function of date of treatment.

It would seem that air temperature would have an effect on herbicidal effectiveness simply because volatility and evaporation of the herbicide would be increased as temperature increased. Differences in humidity would not affect volatility, but might affect the physiologic condition of plants. There are few references to the effect of humidity on herbicidal effectiveness. Elle (4) found greater effectiveness at low relative humidity; Fisher, et al. (5) found that humidity did not appear to influence herbicidal effectiveness in aerial applications on mesquite.

Relation of carbohydrate content of root xylem to susceptibility.

Total sugar increased gradually until mid-June and then leveled off (Figure 4). Sucrose was the only sugar detected. Both the type of

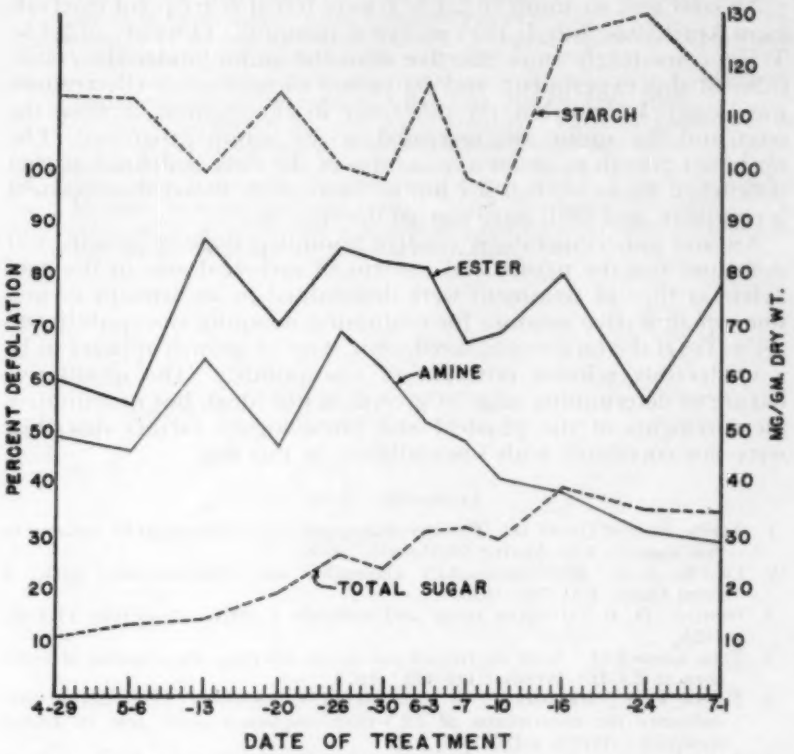

Figure 4. Total sugar and starch contents of root xylem and their relation to defoliation with an ester and an amine of 2,4,5–T.

sugar and the concentration trend corroborated earlier work on mesquite except for a July 2, 1953 reading that revealed a small amount of fructose in the root xylem in addition to the predominant sucrose. Not all fluctuations in starch content before June 10 are significant, although the buildup after that date very likely is significant.

Correlation analyses for starch and total sugar concentrations with defoliation by the ester and the amine salt were not significant when all treatment dates were considered. When the treatment dates from May 26 through July 1 were used, however, there was a significant negative correlation between total sugar concentration and defoliation with the amine salt. Despite this significance, it is not valid to assume that the increase in total sugar concentration is responsible for the decreasing effectiveness of the amine salt. De-

creased absorption as a result of cuticle development is a more likely explanation.

SUMMARY

An ester and an amine of 2,4,5–T were tested at frequent intervals from April 29 to July 1, 1955 on velvet mesquite. The ester of 2,4,5–T was consistently more effective than the amine under the conditions of this experiment, and its period of maximum effectiveness was longer. In addition, the difference in effectiveness between the ester and the amine salt increased as the season progressed. The optimum growth stage for application of the ester is defined as that time when leaves are full size but still succulent, flower development is complete, and pods have started developing.

Air and soil temperature, relative humidity, stage of growth, leaf moisture, and the nature and content of carbohydrates in the root xylem at time of treatment were determined in an attempt to find some quantitative measure for evaluating mesquite susceptibility to 2,4,5–T. Of the factors measured, only stage of growth appears to be a moderately reliable criterion of susceptibility. The qualitative nature of determining stage of growth is not ideal, but quantitative measurements of the physical and physiological factors described were not correlated with susceptibility in this test.

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Effect of Gibberellic Acid on Absorption, Translocation, and Degradation of 2,4–D in Red Kidney Bean

FLOYD M. ASHTON[1]

THE absorption and translocation of 2,4–D (2,4–dichlorophenoxyacetic acid) has been extensively studied by numerous investigators and a number of reviews have been written (3, 7, 8). The methods used in these studies have been critically evaluated (1, 5, 8) and the general conclusion is that in the study of the absorption and translocation of biologically unstable organic compounds, such compounds should be isolated and identified.

It is generally accepted that 2,4–D is a more effective herbicide for plants which are growing rapidly than for those growing slowly. This increased effect is thought to be related to an increase in translocation. The dramatic growth promoting properties of gibberellic acid suggest that it might increase the effectiveness of 2,4–D. It has been reported that pre-treatment of red kidney beans with gibberellic acid 48 hours before 2,4–D application to a single primary leaf increased the amount of 2,4–D found in the untreated areas of the plant three and five days after the 2,4–D application (2). The paper also reports a callus formation at the growing point of the gibberellic acid treated plant which was absent in the untreated plant.

The present paper reports a continuation of this latter study to elucidate the mechanism of the interaction of gibberellic acid and 2,4–D.

MATERIALS AND METHODS

Uniform red kidney bean plants *(Phaseolus vulgaris* L.) with the first trifoliate leaf just starting to unfold were selected for the experimental material. One-half of the plants were sprayed to "runoff" with a 100 ppm aqueous solution of the potassium salt of gibberellic acid[2] 48 hours prior to the 2,4–D application. Twenty-four hours after the gibberellic acid application no increased elongation was observed; 48 hours after, a slight increase in elongation was noted. In all five experiments reported, the plants were treated as above but varied in other details as described below.

Absorption and translocation experiment I.

Ten micrograms of radioactive 2,4–D*[3] in lambda of 95 per cent ethyl alcohol were spread over the entire surface of one primary leaf of the bean plant. The 2,4–D* was labeled with C^{14} in the carboxyl position and had a specific activity of 0.47 millicurie per millimole. The plants were harvested 24, 72, and 120 hours after the 2,4–D* application. The 2,4–D* treated leaves were excised from the plants at the base of the petiole and discarded. The soil was washed from the roots and the plants rapidly killed by placing them in boiling

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[2]Gibberellic acid supplied gratis by Merck and Company, Inc.
[3]The asterisk denotes radioactivity.

80 per cent alcohol. The 2,4–D* was isolated as pure 2,4–D* and quantitatively determined by a previously reported method (1). The experiment was replicated four times.

Absorption and translocation experiment II.

Ten micrograms of 2,4–D* in 10 lambda of 95 per cent alcohol were applied to one primary leaf of the bean plant as two five lambda drops, one drop on each side of the midrib near the base of the leaf. The 2,4–D* was labeled with C^{14} in the carboxyl position and had a specific activity of 1.24 millicuries per millimole. The plants were harvested 24, 72, and 120 hours after the 2,4–D* application. The treated leaves were excised from the plants at the base of the petiole and discarded. The soil was washed from the roots and the plants killed and dried by placing them in a forced draft oven at 60° C. for 24 hours. The plants were weighed and then ground in an intermediate Wiley mill using a 40 mesh screen. The ground plant material was quantitatively extracted with 80 per cent alcohol. The 2,4–D* was isolated as pure 2,4–D* and quantitatively determined by a previously reported method (1). Six plants were used for each treatment time and treatment and combined for the analysis.

Absorption and translocation experiment III.

Ten micrograms of 2,4–D* in 30 lambda of 25 per cent alcohol were applied to a 10 cm^2 area in the center of the upper surface of one primary leaf of the bean plant. The 2,4–D* was labeled with C^{14} in the carboxyl position and had a specific activity of 1.24 millicuries per millimole. The plants were harvested 1, 3, 6, and 10 days after the 2,4–D* application. The 2,4–D* treated leaves were excised from the plants at the base of the petiole and discarded. The soil was washed from the roots and the plants rapidly killed by placing them in boiling 80 per cent alcohol. The 2,4–D* was isolated as pure 2,4–D* and quantitatively determined by a previously reported method (1).

Short time absorption-translocation.

A translocation experiment of relatively short duration (24 hours) was conducted to avoid the complicating problem of 2,4–D degradation. Jaworski and Butts (9) reported that 24 hours after a primary leaf of a bean plant was treated with carboxyl labeled 2,4–D*, 81 per cent of the total radioactivity of the stem was 2,4–D*; however, at 96 hours only 40 per cent was 2,4–D*. Ten micrograms of 2,4–D* in 10 lambda of 20 per cent ethyl alcohol were applied to a 6 to 8 cm^2 area in the center of the upper surface of one of the primary leaves of the bean plant. The 2,4–D* was labeled with C^{14} in the carboxyl position and had a specific activity of 0.47 millicurie per millimole. The plants were harvested at 0.5, 1.0, 2.0, 4.0, and 24.0 hours after the 2,4–D* application. The 2,4–D* treated primary leaf was excised from the plant at the base of the petiole and discarded. The soil was washed from the roots and the plants killed, dried, and ground as in Experiment II. The cupped plachet preparations of the

ground tissue at infinite thickness were counted with a thin window G-M tube. The counts were converted to microgram equivalents of 2,4–D* per plant. The experiment was replicated four times.

Degradation experiment.

A ten-day study of 2,4–D degradation was conducted by removing the 2,4–D* treated leaves 24 hours after the 2,4–D treatment to avoid the complicating factors of continued absorption and translocation. Ten micrograms of radioactive 2,4–D* in 50 lambda of 20 per cent ethyl alcohol were applied to a 10 cm^2 area in the center of the upper surface of one primary leaf of the bean plant. The 2,4–D* was labeled with C^{14} in the carboxyl position and had a specific activity of 0.47 millicurie per millimole. The 2,4–D* treated leaves were excised from the plants at the base of the petiole 24 hours after the 2,4–D* application and discarded. The plants were harvested 1, 3, 5, 7, and 10 days after the 2,4–D* application. The soil was washed from the roots and the plants killed and dried by placing them in a forced draft oven at 60° C. for 24 hours. The plants were weighed and then ground in an intermediate Wiley mill using a 40-mesh screen. The dry ground plant tissues were extracted with 3 successive 10 ml portions of 80 per cent ethyl alcohol. The 2,4–D* was isolated as pure 2,4–D* and quantitatively determined by a previously reported method (1). The experiment was replicated four times.

RESULTS AND DISCUSSION

The results of the first three experiments are presented in Table 1. The amount of 2,4–D* in the nontreated areas of the plants decreased with time in both the gibberellic acid-treated plants and the untreated plants after the first day. Evidently, the rate of degradation exceeded the rate of absorption and translocation. However,

Table 1. Amount of 2,4–D in the whole plant, minus the treated leaf, as a percent of that applied.

Days after appl.	Experiment I		Experiment II		Experiment III	
	GA treated	Untreated	GA treated	Untreated	GA treated	Untreated
1	4.8±1.0	4.6±1.1	10.6*	13.0*	5.0±0.6	3.2±1.4
3	3.1±0.7	1.8±0.4	6.2*	2.8*	6.4±2.4	1.6±1.2
5	2.7±0.9	0.9±0.3	5.0*	1.6*	———	———
6	———	———	—	—	1.0±0.2	1.8±0.8
10	———	———	—	—	1.2±0.4	0.6±0.2

*Six plants were used for each treatment and these were combined for the analysis, therefore standard deviations are not available.

this decrease in the amount of 2,4–D* was substantially less in the gibberellic acid-treated plants than in the non-treated plants at three and five days after the 2,4–D* application. Therefore, the gibberellic acid-treated plants contained more 2,4–D* than those that did not receive gibberellic at these periods of time. This substantiates the previous work (2).

However, this difference appears to be of short duration as indi-

cated by experiment III, which extended over 10 days compared with 5 days, as in experiments I and II. Apparently the rate of degradation exceeded the rate of absorption and translocation to the extent that in both the gibberellic acid-treated plants and in those that did not receive gibberellic acid, the 2,4–D* level was reduced to a relatively low uniform value at six to ten days. It is probable that the actual rate of translocation and degradation of 2,4–D* was greater in experiment III than in experiments I and II because of higher light intensities and temperatures of the greenhouse during experiment III. The increased amount of 2,4–D* in the gibberellic acid treated plants is in agreement with the generalization that 2,4–D is most effective in plants which are growing rapidly.

The amount of 2,4–D in the untreated areas of a plant is the result of at least three distinct physiological processes. These are absorption, translocation, and inactivation. Inactivation probably results from a degradation of the 2,4–D molecule into smaller units, but factors of physical adsorption and complexing with cellular constituents should not be ignored.

The short time absorption-translocation experiment was conducted to study these two processes with minimum degradation. The degradation of 2,4–D* during this 24 hour experimental period probably amounted to about 19 per cent (6). This indicates that absorption-translocation was the primary processes being studied. Figure 1 clearly demonstrates that the increased level of 2,4–D* in the gibberellic acid-treated plants is not the result of absorption-translocation during this 24 hour period.

The degradation experiment was conducted to study degradation in the absence of absorption and translocation. The removal of the

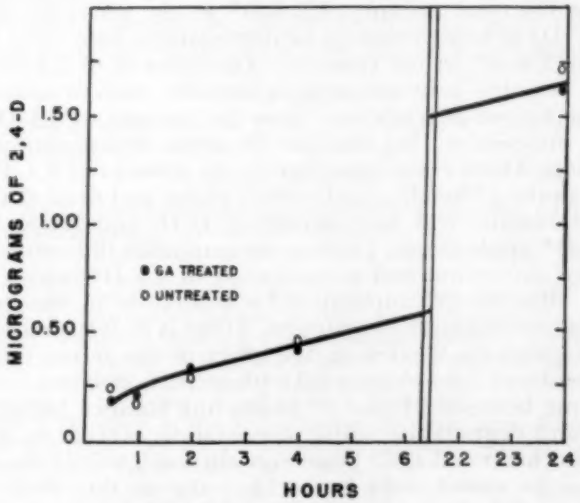

Figure 1. Effect of gibberellic acid on "short time absorption-translocation" of 2,4–D. No effect.

2,4–D* treated leaf eliminated further absorption and translocation. The data from this experiment (Figure 2) clearly indicate that increased level of 2,4–D* in the gibberellic acid treated plants is not due to a decrease in the degradation of 2,4–D*.

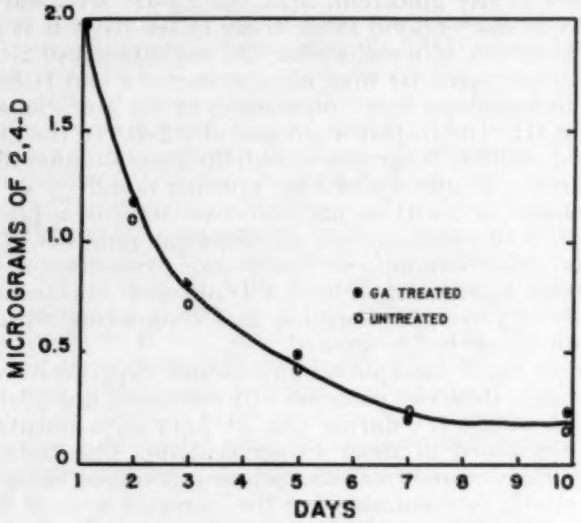

Figure 2. Effect of gibberellic acid on degradation of 2,4–D. No effect.

The amount of 2,4–D* in the untreated areas of the plant can be considered as a reservoir into which 2,4–D* is being translocated, increasing the total amount of 2,4–D* in the reservoir; and from which 2,4–D* is being removed by degradation, decreasing the total amount of 2,4–D* in the reservoir. The amount of 2,4–D* in this reservoir remains constant only when the rate of translocation equals the rate of degradation. Since the amount of 2,4–D* in the reservoir increases during the first 24 hours, translocation exceeds degradation. There is no difference in the amount of 2,4–D* in the reservoir in the gibberellic acid-treated plants and those that did not receive gibberellic acid in experiments I, II, and III at 24 hours after 2,4–D* application. There is no detectable difference between the rate of absorption and translocation of 2,4–D* during the first 24 hours after 2,4–D* application, as evidenced in the short time absorption-translocation experiment. There is no difference in degradation at 24 hours. Therefore, the effect of the interaction of gibberellic acid and 2,4–D* must take place after 24 hours.

Sometime between 24 and 72 hours, the balance between translocation and degradation shifts; degradation exceeds translocation since plants harvested at 72 hours contain less 2,4–D in the reservoir than those harvested at 24 hours. It is also in this 24 to 72 hour period that the effect of the interaction of gibberellic acid and 2,4–D was detected, which results in a larger amount of 2,4–D in

the reservoir of the gibberellic acid-treated plant than in those that did not receive gibberellic acid. As previously pointed out, an increase in the amount of 2,4–D in this reservoir can result from a decrease in degradation or an increase in translocation. The first possibility has been experimentally eliminated. Therefore, logically, the rate of translocation of 2,4–D in the gibberellic acid-treated plant must exceed that of those that did not receive gibberellic acid. This would be extremely difficult to prove experimentally with a compound as labile as 2,4–D.

Therefore, it is concluded that the increased amount of 2,4–D in the reservoir of the gibberellic acid-treated plant after 24 hours results from 2,4–D being translocated at a relatively higher rate in the gibberellic acid-treated plant than in those that did not receive gibberellic acid. This may represent a qualitative or quantitative reduction of the complexing of 2,4–D with the cellular constituents of the treated leaf.

SUMMARY

The previous work (2) reporting that pre-treatment of red kidney beans with gibberellic acid 48 hours before 2,4–D* application to a single primary leaf increased the amount of 2,4–D* found in the untreated areas has been substantiated. This differential disappears after several days when the level of 2,4–D* in both the gibberellic acid plants and those not receiving gibberellic acid is reduced to a relatively low value, due to the degradation of 2,4–D*.

The rate of absorption and translocation of 2,4–D* during the first 24 hours after 2,4–D* application does not appear to be affected by gibberellic acid.

The rate of degradation of 2,4–D* does not appear to be affected by gibberellic acid.

The increased amount of 2,4–D* in the gibberellic acid-treated plants after 24 hours apparently results from increased translocation.

ACKNOWLEDGMENTS

The technical assistance of T. R. Braun and K. Dunster is gratefully acknowledged.

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Effects of Soil Type and Time on the Herbicidal Activity of CDAA, CDEC, and EPTC[1]

T. J. SHEETS[2]

RECENT investigations on the use of 2–chloro–*N*,*N*–diallylacetamide (CDAA), 2–chloroallyl diethyldithiocarbamate (CDEC), and ethyl *N*,*N*–di–*n*–propylthiolcarbamate (EPTC) for weed control have stressed soil incorporation rather than the more conventional application to the soil surface. By this treatment loss of the herbicides as vapors may be reduced, the risk of crop injury lessened, and weed control improved. Although both pre-planting and pre-emergence soil incorporation have been investigated, pre-planting treatments appear most promising. In many weed-crop situations the results obtained with EPTC have been especially promising.

When herbicides are incorporated into the soil rather than applied to the surface, the influence of the soil on herbicidal activity may be greatly altered. The herbicide molecules are in more intimate contact with the soil particles. Generally the chemicals are in soil containing more moisture. They are subjected to air containing less oxygen and more carbon dioxide. They are exposed to much less sunlight. Vapors which form are subjected to virtually no air movements. The temperature of the soil below the surface varies less than that of the surface layer. These factors probably affect the behavior of herbicides.

Reports of research in which the influence of soil type on the activity of CDAA and CDEC has been critically evaluated are lacking. Hannah (2) suggested that organic matter and clay may prevent rapid leaching of these two herbicides. This observation was based on field tests in which weeds were controlled for longer periods following treatment on soils containing more organic matter and clay than on soils containing less of these components. Hannah further stated that the chloroacetamide herbicides were reversibly adsorbed, and he suggested that rapid adsorption prevented rapid leaching. The work of Otten et al. (3) indicated that microorganisms are involved in the loss of CDEC activity from soils. CDEC was inactivated more rapidly at 25° C than at 8° C, and the rate of degradation increased as the soil moisture content increased from that of air dry soil to that of soil at the moisture equivalent. The same investigators showed that CDAA was leached more readily by simulated rain than CDEC.

The influence of soil type on the initial phytotoxicity of EPTC has been reported (1). Soil type markedly influenced the activity of EPTC. The lower activity of EPTC in clay and clay loam soils was

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[1]Cooperative investigations of the Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture and the California Agric. Exp. Station.

[2]Research Agronomist, Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, Botany Department, University of California, Davis.

apparently caused by greater adsorption of the herbicide. Furtick[3] suggested that the residual life of EPTC was very short, and prolonged weed control by this chemical was attributed to killing of weed seeds rather than to active residual chemical.

The tests reported herein were designed (a) to determine the variation in the herbicidal activity of CDAA, CDEC, and EPTC in several soil types, (b) to study the influence of the clay and organic matter contents, cation exchange capacity, and pH of the soils on herbicidal activity, and (c) to study the inactivation of the herbicides in the several soil types. The data in this paper are significant in view of the present emphasis in research on soil incorporation of these herbicides.

EXPERIMENTAL PROCEDURE

The soils used in this experiment were collected in California and Arizona. They were chosen for their agricultural importance and for convenience in collecting and transporting to the laboratory. Certain soil properties are recorded in Table 1.

Table 1. Percent organic matter, percent clay, cation exchange capacity, and pH of soils.

Soil type[a]	Per cent organic matter	Per cent clay	Cation exchange capacity[b]	pH
Sacramento clay–1	4.83	79.5	——	5.7
Sacramento clay–2	5.96	78.4	31.2	7.2
Yolo silty clay	2.44	45.0	25.7	8.0
McClellan clay	1.39	32.4	19.8	8.4
Yolo clay loam	2.43	38.7	——	7.2
Yolo sandy clay loam	1.29	22.5	13.9	7.1
Hesperia sandy loam	.28	16.0	7.5	7.5
McClellan loam	1.13	22.7	14.2	8.1

[a]Sacramento clay–1 was used with EPTC. Sacramento clay–2 was used with CDAA and CDEC.
[b]Milliequivalents per 100 g oven dry soil.

The toxicity of CDAA, CDEC, and EPTC to oat plants in seven soil types was investigated under greenhouse conditions. Methods of preparing the soils have been reported (4).

Portions of soil, each weighing 500 g on an oven-dry basis, were placed in No. 2 metal cans. The cans did not drain; hence no herbicides were lost by leaching. The herbicides[4] were added to the soils as water emulsions. The emulsion for each culture was prepared by mixing the appropriate amount of chemical from a stock emulsion with sufficient water to wet the soil to field capacity. Approximately uniform distribution in the soil was obtained by adding the suspension in three portions to three portions of the soil as the metal cans were filled. The concentrations were 0, 2.5, 5.0, 10, 20, 40, 80, and 160 ppmw. In addition three lower concentrations (0.31, 0.62, and

[3]Furtick, W. R. Paper presented at the Western Weed Control Conf. Boise, Idaho. Mar. 20–21, 1957.

[4]The experimental samples of CDAA and CDEC were supplied by Monsanto Chemical Company, St. Louis, Missouri and the sample of EPTC by Stauffer Chemical Company, Mountain View, California.

1.25 ppmw) were prepared for EPTC. Immediately after treatment 13 oat seeds were planted per can. The plants were watered at least daily during the growing period. Fresh weights of the plant parts above the soil level were taken 28 days after seeding. The cultures were allowed to remain unwatered for the next 28 days. At the close of the dry period the soil in each can was pulverized, and the plant residue from the previous harvest was returned to the container. The soil in each can was wet to field capacity and seeded again. This system of cropping was continued for a total of three crops with CDAA and CDEC cultures and five crops with EPTC cultures. The experimental design was a randomized split block with three replications.

For analysis and presentation, part of the data was converted to ED_{50} values. The ED_{50} (50 percent effective dosage) represents the concentration of herbicide in ppmw which reduced growth 50 percent. These values were obtained by plotting the fresh weight as percent of the control against the logarithm to the base 10 of the concentration and constructing a freehand curve to fit the points. The point on the concentration axis which corresponded with the point of intersection of the curve and the 50 percent yield level was the ED_{50} as a logarithm. The antilogarithms of the 50 percent points gave the ED_{50} in ppmw. ED_{50} values for CDAA and CDEC were determined for the initial crop only. For EPTC ED_{50} values were determined for all crops.

RESULTS AND DISCUSSION

Although the concentrations of herbicides are recorded in ppmw, a rough conversion to lb/A is available. Assuming an acre foot of soil to weigh 4 million pounds and further assuming the herbicides to be mixed with the entire acre foot, we can multiply by four and convert ppmw to lb/A. If we consider the herbicides to be mixed with only ¼ of the acre foot or 3 in of soil, our ppmw concentration is roughly equal to lb/A.

The influence of soil type on the ED_{50} values of CDAA and CDEC for the first crop is shown in Table 2. Between the two extreme soil types there was a 2.9 and a 2.7 fold difference in effective concentration for CDAA and CDEC respectively. In all soils the concentration

Table 2. The influence of soil type on the phytotoxicity of CDAA and CDEC the first cropping as shown by ED_{50} values for oats.

Soil type	ED_{50} value (ppmw)	
	CDAA	CDEC
Sacramento clay-2	8.6 a	17.0 bc
Yolo silty clay	3.4 e	9.0 d
McClellan clay	6.9 b	12.4 bcd
Yolo clay loam	5.7 c	24.3 a
Yolo sandy clay loam	4.4 d	15.0 bcd
Hesperia sandy loam	3.0 e	10.6 cd
McClellan loam	8.4 a	19.8 ab

*Means followed by the same letter or letters do not differ significantly at the 5 per cent level of probability.

of CDEC required to produce a 50 percent reduction in the yield of oat plants was higher than that of CDAA. Although the herbicidal activity varied among soil types, there appeared to be no simple relation between activity and the soil factors of Table 1.

During the last 7 days of the initial crop, the plants on some of the CDAA and CDEC cultures began to recover from the early injury. For this reason the ED_{50} values in Table 2 may be slightly greater than the true initial toxicity values.

One of the primary aims in these experiments was to study soil inactivation of the herbicides. In the experiments of Otten et al. (3) the activity from 8 lb/A of CDEC applied to the soil surface disappeared within 5 days in moist soil at 25° C. There was no loss of CDEC in air-dry soil in 47 days. The data in Table 3 show that high

Table 3. Fresh weights of oats above the soil level at four concentrations of CDAA and CDEC for two croppings.

Soil type	Conc. ppmw	Fresh weight as per cent of the control			
		CDAA		CDEC	
		Crop 1	Crop 2	Crop 1	Crop 2
Sacramento clay–2.....	20	9[a]	106	44[a]	94
	40	3[a]	100	22[a]	90
	80	0	106	3[a]	68
	160	0	82	0	32[a]
Yolo silty clay.....	20	11[a]	91	51[a]	63
	40	2[a]	95	17[a]	86
	80	0	100	9[a]	85
	160	0	114	0	57[a]
McClellan clay.....	20	3[a]	103	34[a]	97
	40	1[a]	94	5[a]	92
	80	0	87	1[a]	76[a]
	160	0	3[a]	0	20[a]
Yolo clay loam.....	20	17[a]	116	61[a]	71
	40	3[a]	113	38[a]	84
	80	0	110	10[a]	67[a]
	160	0	67[a]	0	22[a]
Yolo sandy clay loam....	20	13[a]	90	52[a]	85
	40	6[a]	95	12[a]	97
	80	0	103	3[a]	61[a]
	160	0	21[a]	0	25[a]
Hesperia sandy loam.....	20	4[a]	103	30[a]	84
	40	0	99	11[a]	97
	80	0	55[a]	0	49[a]
	160	0	8[a]	0	4[a]
McClellan loam.....	20	18[a]	105	59[a]	78
	40	1[a]	96	18[a]	95
	80	0	89	3[a]	69[a]
	160	0	29[a]	0	19[a]

[a]Symptoms of herbicidal injury recognizable.

concentrations of CDAA and CDEC were reduced to innocuous levels in all soils within 3 months. For this experiment the temperature was warm continuously, and during 2 of the 3 months soil moisture was adequate for the growth of oats. Accumulation of soil residues probably would not occur from repeated annual applications of these herbicides at rates used for selective weed control in crops.

The initial phytotoxicity of EPTC in the various soil types is shown in the first column of Table 4. Low concentrations of EPTC

Table 4. The influence of time on the herbicidal activity of EPTC as shown by ED_{50} values for oats.

Soil type	ED_{50} value (ppmw)					
	Crop 1	Crop 2	Crop 3	Crop 4	Crop 5	Crop 5 minus Crop 1
Sacramento clay-1	1.6	5.2	8.3	15.1	18.3	16.7
Yolo silty clay	1.2	11.7	30.7	68.5	96.4	95.2
McClellan clay	0.5	4.6	14.7	34.7	63.3	62.8
Yolo clay loam	0.9	3.6	8.2	17.3	18.8	17.9
Yolo sandy clay loam	0.4	3.8	12.0	25.3	49.9	49.5
Hesperia sandy loam	0.2	2.6	19.8	69.7	103.	103.
McClellan loam	0.5	5.6	19.3	36.7	63.7	63.2

reduced the growth of oats 50 percent. Much higher concentrations of CDAA and CDEC were required. On a percentage basis, the variation in CDAA and CDEC activity among soil types was less than the variation of EPTC activity among soil types. In absolute amounts, however, CDAA and CDEC activity varied most. These results indicate that as a soil-incorporated herbicide EPTC should be more efficient than CDAA and CDEC.

There was a very close relation between EPTC toxicity to the first crop and percent organic matter, percent clay, and cation exchange capacity (1). However organic matter, percent clay, and cation exchange capacity were very closely related. For example, soils high in organic matter tended to be high in clay; the same soils had high cation exchange capacities.

The influence of time on the herbicidal activity of EPTC is shown in Table 4. EPTC was more persistent than CDAA and CDEC. The amount of EPTC inactivated during the first 3 months was small in most soils. In Yolo clay loam, Yolo sandy clay loam, and Hesperia sandy loam less than 4 ppmw (or 4 lb/A assuming soil incorporation to a depth of 3 in) reduced the fresh weight of oats 50 percent at the end of the second cropping (3 months). During two of these months soil temperature and soil moisture were presumably favorable for microbial degradation. Since the values in Table 4 are concentrations which reduced growth 50 percent, lower dosages caused injury. Although EPTC may remain active for 3 or 4 months at rates used for weed control, there appears to be no danger of accumulation from repeated annual applications.

ED_{50} values for EPTC from four crops in autoclaved and unautoclaved Yolo silty clay are shown in Table 5. The soil was autoclaved prior to addition of the herbicide. Yolo silty clay was chosen for this test because EPTC activity disappeared very rapidly from it (Table 4). The data in Table 5 show that autoclaving the soil prior to treatment greatly retarded inactivation of EPTC. Although EPTC is quite volatile (1), these data suggest that microbial breakdown may be a major pathway of EPTC inactivation when the chemical is

Table 5. The influence of time and autoclaving the soil (Ysc) prior to treatment on the herbicidal activity of EPTC as shown by ED_{80} values for oats.

Treatment	ED_{80} value (ppmw)			
	Crop 1	Crop 2	Crop 3	Crop 4
Soil autoclaved prior to adding herbicide	1.0	3.7	6.4	19.5
Soil not autoclaved	1.2	9.5	31.0	70.5

incorporated in the soil. Therefore EPTC persistence may be greatly prolonged in the field when conditions for microbial growth are less favorable. During the late fall, winter, or early spring, the soil temperature usually limits microbial activity. The moisture content of the soil is often sufficient during the same period to inhibit aerobic microbes. These observations suggest that toxic concentrations of EPTC, when it is incorporated into the soil, may remain for 2 to 3 months under some conditions even at application rates of 2 to 5 lb/A. Oats are very susceptible to EPTC, and consequently they may be injured by concentrations which do not affect more resistant plants. Nevertheless critical studies under field conditions are needed to determine the interval required between the time of pre-planting applications of EPTC and the time crop seeds are planted.

In the tests described in this paper, drainage was restricted. This experimental condition would tend to minimize differences among soil types caused by the effects of moisture and aeration on microbial decomposition. Loss of herbicides by leaching was completely eliminated. Both of these factors, which are related to soil type, would probably affect the activity of herbicides under field conditions.

SUMMARY

The effects of soil type and time on the herbicidal activity of CDAA, CDEC, and EPTC were investigated under greenhouse conditions. In the discussion of the results, soil incorporation of herbicides was emphasized. The results may be summarized as follows:

Between the two extreme soil types there was a 2.9 and 2.7 fold difference in effective concentration for CDAA and CDEC respectively. In all soils a higher concentration of CDEC than CDAA was required to produce the same degree of injury. High concentrations of CDAA and CDEC were reduced to innocuous levels in 1 to 3 months.

There was a very close relation between the initial toxicity of EPTC and percent organic matter, percent clay, and cation exchange capacity. EPTC was inactivated much more slowly in all soils than CDAA and CDEC. In some soils less than 4 ppmw was toxic to oats after 3 months. EPTC was inactivated about ⅓ as rapidly in autoclaved as in unautoclaved soil. This may implicate microbial breakdown as a major pathway of EPTC loss from soils. EPTC activity may be greatly prolonged under field conditions unfavorable for growth of soil microorganisms.

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Rotary Hoeing in Combination with Herbicides and Other Cultivations for Weed Control in Soybeans[1]

ELROY J. PETERS, DAYTON L. KLINGMAN, and RUSSELL E. LARSON[2]

EARLY patent records show that a rotary-hoe type of implement has been in existence since 1839 (2). The rotary hoe was used on soybeans as early as 1915, and important considerations for weed control were discussed in "Soybeans in Indiana, 1928," (1) but its use did not become widespread until about 1929.

Scientific literature on the use of the rotary hoe is sparse and consequently recommended uses of the rotary hoe as a cultural tool have not been well defined by published research data. Few experimental data are available on the speed of operation or on the soil conditions necessary for satisfactory operation of the rotary hoe (4). It has been thought that the surface soil must be dry and that high speeds are necessary.

Experiments, therefore, were designed to evaluate the effectiveness of rotary hoeing at various stages of weed growth when soil was wet and dry and in combinations with pre-emergence chemical treatments. Weed-control methods were evaluated on soybeans planted in rows as well as on soybeans drilled in solid stands.

MATERIAL AND METHODS

Soybeans planted in rows.

The effects of various conditions and times of using the rotary hoe for weed control were evaluated in combination with other cultivations and supplemental weed-control treatments from 1955 to 1957. The experiments were laid out in split-plot designs with rotary hoeings and other cultivations randomized as main plots (Tables 1 and 2). Sub-plots randomized within main plots consisted of two rows 30 feet long. Treatments of these sub-plots consisted of (a) check (which had no treatment other than specified cultural treatments) (b) handweeding and (c) use of an herbicide. These experiments were replicated three times each year.

"Timely" rotary hoeings were planned to be given when conditions were thought to be optimum with a dry soil surface but with adequate moisture below the surface. The weeds were in the "white" stage, less than ¼ inch high. "Late" rotary hoeing was usually done a week or 10 days after the "timely" rotary hoeings. On a few occasions weather interferred with the "timely" treatments and the "late" treatments were applied within several days of the "timely" treat-

[1]Report of a cooperative study conducted by the Agricultural Research Service, United States Department of Agriculture and the Missouri Agricultural Experiment Station, Columbia, Missouri. The Experiments partly supported by a grant from the National Soybean Crop Improvement Council.

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ments. "Wet" rotary hoeings were done as soon after rains as the soil was dry enough so that mud did not ball up excessively on the rotary hoe. "Timely, wet" treatments were sometimes difficult to apply because wet conditions did not always exist when weeds were at the desired size for rotary hoeing.

The rotary hoe used in this study was pulled at a speed of 4 to 5 mph. The wheels were fabricated of sharpened steel angle teeth riveted to a central plate section. The peripheral diameter of these toothed wheels was 13¾ inches with the tooth tips and wheels spaced at 3 inches. Concrete blocks were used as weights to give what penetration was thought to be necessary. In this paper cultivations refer to the operation of a one-row cultivator equipped with one 7-inch half sweep and two 7-inch full sweeps on each side of the row. A rotary-hoe attachment over the row was added to this cultivator for the first two cultivations of the 4-cultivation treatment included in 1957.

Sub-treatments were similar in all years except that the sodium salt of pentachlorophenol (PCP) at 20 lb/A of active ingredient was substituted in 1956 and 1957 for isopropyl *N*–(3–chlorophenyl) carbamate (CIPC) at 8 lb/A which was used in 1955 (Tables 2 and 3). The herbicides were applied in water at the rate of 40 gpa the day of planting or the day following. The weeds in the hand-weeded plots were removed by pulling and hoeing. Weeds may have exerted some competitive effects on the soybeans because sometimes weeds were 4 to 6 inches tall before they were removed.

The experiments were performed on Mexico silt loam at the University of Missouri Experiment Station at Columbia, Missouri. Perry soybeans were planted at the rate of 40 lb/A on May 17, 1955, and Clark soybeans were planted at the same rate on May 11, 1956 and May 7, 1957. Planting was in 40-inch rows at a depth of 1 to 1½ inches.

One 30-foot row of each plot was harvested and threshed to obtain the yields of soybean seed. In 1956 and 1957 the weeds in each plot were cut and weighed at harvest and the moisture content determined to give the dry weight yields of weeds per acre.

Soybeans planted in solid stand.

Soybeans were planted with a grain drill in 8-inch rows at the rate of 90 pounds per acre. The drilled soybeans were planted on the same dates as the soybeans planted in rows and herbicides were applied within a day following planting. The experiments were laid out as split-plot designs with rotary hoeing as main plots 98 feet long and 7 feet wide. Three sub-plots 30 feet long with 4-foot alleys were laid out in each whole plot.

The rotary-hoe equipment, its use and the methods of applying the herbicides were the same as those described in connection with soybeans planted in rows. The treatments applied in 1956 and 1957 are shown in Table 4.

Sub-plots in 1956 consisted of (a) check, (b) pre-emergence treatment with 7½ lb/A of the amine salt of 4,6–dinitro–*o*–*sec*–butyl-

phenol (DNBP) and (c) pre-emergence treatment with 20 lb/A of the sodium salt of PCP. DNBP injured soybeans severely and was replaced with 2–chloro–*N*,*N*–diallylacetamide (CDAA) at 4 lb/A in 1957. Other sub-treatments were repeated in 1957.

Weeds and soybeans were counted within two 2 x 4-foot quadrats placed at random in each plot, after completion of the rotary-hoe treatments. When the soybeans were ripe, a strip 30 inches wide and 30 feet long was mowed from the center of each plot. The weeds were separated from the soybeans and weighed. Moisture content of the weeds was determined and the dry weight of the weeds per acre was calculated. The soybeans were threshed and yields per acre were determined.

RESULTS

Soybeans planted in rows.

The yields of soybeans in 1955 were significantly reduced by repeated rotary hoeing until the soybeans had reached 8 inches in height (Table 1). All other rotary-hoe treatments plus one cultivation produced higher yields than one cultivation alone. The timely use of the rotary hoe under both wet and dry conditions produced higher yields than using it later under the same conditions.

Table 1. Yields of soybeans planted in rows as affected by cultural and pre-emergence herbicide treatments, 1955.

Cultural treatments	Yields resulting from sub-treatments, bu/A			
	Check	Hand-weeding	8 lb/A CIPC	Average
1 cultivation	21.1	28.9	24.8	24.9
3 rotary hoeings (timely) plus 1 cultivation	28.9	34.0	23.8	28.9
2 rotary hoeings (late) plus 1 cultivation	24.8	33.3	22.4	26.9
3 rotary hoeings (wet, timely) plus 1 cultivation	31.7	32.8	24.5	29.7
2 rotary hoeings (wet, late) plus 1 cultivation	17.1	34.8	26.2	26.2
4 rotary hoeings (timely) until beans were 8 inches tall	14.0	26.0	19.1	19.7
Average	23.0	31.6	23.5	
LSD (5% level): Cultural treatments		4.6		
Sub-treatments		6.9		

In hand-weeded plots the weeds were removed from an 8- to 10-inch band over the row. The single cultivation failed to remove all the weeds between the rows; therefore they were removed by hand-hoeing 6 weeks after planting. Although these weeds between the rows may have exerted some competitive effects on the soybeans, hand-weeded plots yielded significantly more soybeans than the check plots or those treated with 8 pounds of CIPC.

Excellent weed control was obtained with CIPC but rather severe stunting injury of the soybeans occurred. In main plots given one cultivation, only the sub-treatments with CIPC resulted in increased yield over the check plots. Where soybeans were rotary-hoed until they were 8 inches tall CIPC also resulted in increased yield over the check. The reason may have been that CIPC-treated soybeans were considerably smaller than the untreated ones at the last rotary

hoeing and were injured less by the hoeing, or that the weed control resulting from CIPC allowed the soybeans to recover more fully than in plots with inferior weed control.

Rotary hoeing and cultivation did not significantly affect the yields of soybeans in 1956 or 1957 (Table 2). In plots having no herbicide treatment poorer yields in both years resulted from four rotary hoeings continued until the beans were 8 inches tall than from most other cultural treatments. "Timely" rotary hoe treatments were about equal under both "wet" and dry conditions when averaged for the years 1955, '56 and '57 (Tables 1 and 2). The 3-year average of the check sub-treatment was 32.3 bu/A for the "timely" rotary hoeing treatment and 32.4 bu/A for the "timely, wet" treatment. Late rotary hoeing in 1956 resulted in reduced yields. There was practically no difference between yields from plots cultivated two or three times.

Soybean yields on plots treated with PCP, supplemental to cultural treatments, average 35.8 bu/A, and those having cultural treatments alone averaged 31.7 bu/A in 1956 and 1957 (Table 2). This is an average increase of 4.1 bu. for pre-emergence treatments with PCP. Yields from plots given PCP treatments averaged only 1 bu/A less than hand-weeded plots. The use of PCP or hand-weeding tended to eliminate differences due to cultivation treatments.

Table 2. Yields of soybeans planted in rows as affected by cultural and pre-emergence herbicide treatments, 1956 and 1957.

Cultural treatments	Yields resulting from sub-treatments, bu/A								
	1956				1957				1956–57
	Check	Hand-weeding	20 lb/A PCP	Av.	Check	Hand-weeding	20 lb/A PCP	Av.	Av.
2 cultivations	24.0	30.7	31.6	28.8	37.7	46.0	41.0	41.6	35.2
3 cultivations	27.9	31.0	28.7	29.2	40.2	46.0	43.8	43.3	36.2
2 rotary hoeings (timely) plus 2 cultivations	28.5	30.1	32.0	30.2	39.4	41.2	37.9	39.5	34.8
2 rotary hoeings (wet, timely) plus 2 cultivations	31.3	31.8	28.7	30.9	34.1	40.9	41.3	38.7	34.8
1 rotary hoeing (wet, timely) plus 2 cultivations	25.5	31.7	30.5	29.2	42.9	44.0	42.8	43.2	36.2
1 rotary hoeing (late) plus 2 cultivations	22.1	27.9	27.0	25.7	34.2	40.9	44.9	40.0	32.8
3 or 4 rotary hoeings (timely until beans were 8 inches tall) plus 1 cultivation	19.0	30.4	30.2	26.2	32.1	45.0	40.9	39.3	32.8
4 cultivations (2 with rotary hoe attachment)	30.8	28.2	28.6	29.2	—	—	—	—	—
Average	26.2	30.2	29.8		37.2	43.3	41.8		
LSD (5% level): Cultural treatments			n.s.				n.s.		
Sub-treatments			1.2				2.6		
Cultural treatments + sub-treatments			3.4				n.s.		

Where no sub-treatments were applied (check sub-plot), rotary hoeing in addition to two cultivations tended to give higher yields than two cultivations alone but was not better than three cultiva-

tions. "Timely" rotary hoeings during both years resulted in better yields than "late" rotary hoeing. Little difference occurred between "timely" rotary hoeings under "wet" and dry conditions. Four rotary hoeings (until the beans were 8 inches tall) resulted in the lowest yields.

Pre-emergence treatments with PCP in combination with two or three cultivations were much more effective in controlling weeds than were combinations of rotary hoeing and cultivations with no herbicide treatment (Table 3). There was no advantage in the use of the rotary hoe in addition to pre-emergence PCP treatments in 1956, and in 1957 the effectiveness of PCP was slightly reduced by rotary hoeing.

Table 3. Yields of weeds (dry matter) in soybeans planted in rows as affected by cultural and pre-emergence herbicide treatments, 1956 and 1957.

Cultural treatments	Yields resulting from sub-treatments, lb/A								
	1956				1957				1956–57
	Check	Hand-weeding	20 lb/A PCP	Av.	Check	Hand-weeding	20 lb/A PCP	Av.	Av.
2 cultivations	1,621	78	38	579	3,322	0	236	1,186	882
3 cultivations	1,204	39	30	424	1,402	0	0	467	446
2 rotary hoeings (timely) plus 2 cultivations	1,294	46	40	460	1,944	0	1,034	986	723
2 rotary hoeings (wet, timely) plus 2 cultivations	1,085	23	53	387	2,804	0	0	935	661
1 rotary hoeing (wet, timely) plus 2 cultivations	1,826	49	50	641	1,461	0	414	625	633
1 rotary hoeing (late) plus 2 cultivations	2,673	96	142	969	3,640	0	389	1,343	1,156
3 or 4 rotary hoeings (until beans were 8 inches tall) plus 1 cultivation	3,329	36	25	1,129	5,137	0	554	1,897	1,513
4 cultivations (2 with rotary-hoe attachment)	511	29	34	191	—	—	—	—	—
Average	1,693	49	51		2,816	0	372		
LSD (5% level): Cultural treatments				n.s.				—	
Sub-treatments				264				—	

"Timely" rotary hoeings under both "wet" and dry conditions controlled weeds better than late rotary hoeings during both years (Table 3).

Soybeans planted in solid stand.

Yields of soybeans in 1956 and 1957 were increased an average of 7.1 bu/A over the check plot by the pre-emergence use of PCP (Table 4).

Plots treated with DNBP in 1956 produced an average of 2.4 bu/A more soybeans than the check plots, although there was serious injury to stands of soybeans. CDAA-treated plots in 1957 averaged only slightly better yields than the check plots, while PCP-treated plots were outstanding, and out-yielded the check plots by nearly 10 bushels.

Table 4. Yields of soybeans planted in solid stands as affected by rotary hoeing and pre-emergence herbicide treatments, 1956 and 1957.

Cultural treatments	Yields resulting from sub-treatments, bu/A								
	1956				1957				1956–57
	Check	7½ lb/A DNBP	20 lb/A PCP	Av.	Check	4 lb/A CDAA	20 lb/A PCP	Av.	Av.
Check	13.6	12.8	16.8	14.4	19.2	24.0	35.7	26.3	20.4
Rotary hoeing (timely)	18.4	20.1	19.4	19.3	24.6	26.7	36.9	29.4	24.4
Rotary hoeing (late)	14.1	16.5	20.0	16.9	25.2	21.0	33.5	26.6	21.8
Rotary hoeing (timely, wet)	17.4	20.2	19.7	19.1	27.0	28.0	33.8	29.6	24.4
Rotary hoeing (timely until beans were 8 inches tall)	16.6	20.4	21.1	19.4	28.0	25.6	33.1	28.9	24.2
Rotary hoeing (large wheel, timely until beans were 8 inches tall)	12.3	17.0	23.1	17.5	—	—	—	—	—
Average	15.4	17.8	20.1		24.8	25.1	34.6	28.2	
LSD (5% level): Cultural treatments			n.s.				n.s.		
Sub-treatments			4.5				4.7		

Rotary hoeing generally increased the yields of soybeans over the check (Table 4). "Timely" rotary hoeings were better than "late" rotary hoeings. The latter were only slightly better than the check in 1956 and about equal to the check in 1957. Rotary hoeing was especially beneficial in increasing yields of soybeans on plots receiving no supplemental treatment and on plots receiving DNBP or CDAA. Some benefit occurred from rotary hoeing on PCP-treated plots in 1956 but not in 1957.

The stands of soybeans were only slightly affected by the rotary hoeing (Table 5). Stands of soybeans were reduced significantly by DNBP in 1956 and by PCP in 1957. Although stands were reduced considerably by PCP, yields of soybeans were excellent. Shallow planting with a grain drill probably accounted for the reduction in stand from PCP since no such injury resulted in many other tests with this herbicide where planting was done with a regular planter. Because of frequent injury from DNBP its use was discontinued.

Table 5. Soybean plants as affected by cultural and pre-emergence chemical treatments of soybeans planted in solid stands, 1956 and 1957.

Cultural treatments	Number of soybean plants in 16 sq. feet resulting from sub-treatments								
	1956				1957				1956–57
	Check	7½ lb/A DNBP	20 lb/A PCP	Av.	Check	4 lb/A CDAA	20 lb/A PCP	Av.	Av.
No treatment	86	33	56	58	113	97	62	91	74
Rotary hoeing (timely)	61	29	64	51	92	92	47	77	64
Rotary hoeing (late)	68	23	62	51	97	102	58	86	68
Rotary hoeing (timely, wet)	67	28	52	49	106	103	54	88	68
Rotary hoeing (timely until beans were 8 inches tall)	66	26	54	49	90	105	61	85	67
Rotary hoeing (large wheel, timely, until beans were 8 inches tall)	57	36	49	47	—	—	—	—	—
Average	68	29	56		100	100	56		
LSD (5% level): Cultural treatments			n.s.				n.s.		
Sub-treatments			15.				8		

Pigweeds (*Amaranthus* spp.) and crabgrass (*Digitaria* spp.) were the most numerous weeds present in 1956 and the data show that rotary hoeings were moderately effective in reducing stands of these weeds (Tables 6 and 7). These weeds were predominant again in 1957, but not quite as numerous as in 1956. "Timely" and "timely, wet" rotary hoeings were somewhat better for controlling weeds than the other rotary hoe treatments. On a few occasions, wet weather delayed the "dry, timely" treatments, which consequently were not always applied when weeds were at an optimum size.

Table 6. Pigweed plants as affected by cultural and pre-emergence chemical treatments of soybeans planted in solid stands, 1956 and 1957.

Cultural treatments	Number of pigweed plants in 16 sq. feet resulting from sub-treatments								
	1956				1957				1956–57
	Check	7½ lb/A DNBP	20 lb/A PCP	Av.	Check	4 lb/A CDAA	20 lb/A PCP	Av.	Av.
Check	493	17	14	175	133	54	10	66	120
Rotary hoeing (timely)	219	2	18	80	51	33	1	29	54
Rotary hoeing (late)	142	19	10	57	78	60	1	46	52
Rotary hoeing (timely, wet)	148	6	0	51	52	42	5	33	42
Rotary hoeing (timely until beans were 8 inches tall)	64	2	8	25	68	39	0	35	30
Rotary hoeing (Large wheel, timely until beans were 8 inches tall)	94	7	7	36	—	—	—	—	—
Average	193	9	10		76	46	5		
LSD (5% level): Cultural treatments			31				21		
Sub-treatments			n.s.				20		

Table 7. Crabgrass plants as affected by cultural and pre-emergence chemical treatments of soybeans planted in solid stands, 1956 and 1957.

Cultural treatments	Number of crabgrass plants in 16 sq. feet resulting from sub-treatments								
	1956				1957				1956–57
	Check	7½ lb/A DNBP	20 lb/A PCP	Av.	Check	4 lb/A CDAA	20 lb/A PCP	Av.	Av.
Check	1,760	390	994	1,048	378	67	55	167	608
Rotary hoeing (timely)	333	173	186	231	324	114	46	161	196
Rotary hoeing (late)	271	242	249	254	418	152	29	200	227
Rotary hoeing (timely, wet)	197	148	162	169	342	46	21	136	152
Rotary hoeing (timely until beans were 8 inches tall)	68	53	103	75	349	96	16	154	114
Rotary hoeing (large wheel timely until beans were 8 inches tall)	112	187	90	130	—	—	—	—	—
Average	457	199	297		362	95	33		
LSD (5% level): Cultural treatments			269				n.s.		
Sub-treatments			44				91		

Both DNBP and PCP were effective in reducing the stands of pigweeds and crabgrass (Tables 6 and 7). CDAA also reduced weed stands significantly, but was not as effective as PCP.

The dry weights of weeds in 1956 show that "timely" rotary hoeings under either "wet" or dry soil conditions were more effective in reducing weeds than the late treatments (Table 8). "Timely"

Table 8. Yields of weeds (dry matter) in plots planted to soybeans in solid stands as affected by cultural and pre-emergence herbicide treatments, 1956 and 1957.

Cultural treatments	Yields in lb/A resulting from sub-treatments								
	1956				1957				1956–57
	Check	7½ lb/A DNBP	20 lb/A PCP	Av.	Check	4 lb/A CDAA	20 lb/A PCP	Av.	Av.
Check	3,983	2,575	1,383	2,647	2,368	1,902	116	1,463	2,055
Rotary hoeing (timely)	2,918	629	825	1,458	1,483	733	127	781	1,120
Rotary hoeing (late)	4,655	913	1,358	2,309	806	1,702	149	886	1,598
Rotary hoeing (timely, wet)	2,550	1,213	1,028	1,597	815	566	109	497	1,047
Rotary hoeing (timely, until beans were 8 inches tall)	3,476	343	825	1,548	854	1,404	12	757	1,152
Rotary hoeing (large wheel, timely until beans were 8 inches tall)	3,222	1,269	597	1,696	—	—	—	—	—
Average	3,467	1,157	1,003		1,265	1,261	103		
LSD (5% level): Cultural treatments				n.s.					
Sub-treatments				594					

treatments, continued until the soybeans were 8 inches tall, were less effective in controlling weeds than rotary hoeing discontinued earlier. The more numerous rotary hoeings continued until soybeans were 8 inches tall, injured the soybeans and reduced their competitive effects on the weeds, (check sub-plot).

Both DNBP and PCP reduced weeds to a highly significant degree as compared with the check plot. PCP in particular was more effective than any of the rotary-hoe treatments.

The weed yields in 1957 show that all rotary-hoe treatments reduced the weeds considerably as compared with no rotary hoeing (Table 8). "Timely" rotary hoeings were considerably better than the other rotary-hoe treatments. Wet weather occasionally delayed the dry "timely" treatments, which were thus applied when the weeds were beyond the optimum size.

The weed yields of CDAA-treated plots were little different from those of untreated plots. Weed control on plots treated with PCP was far superior to CDAA. There were only about 100 pounds of weeds per acre compared with more than 1200 for the CDAA treatments and the check plot.

Discussion

These experiments demonstrated the importance of timely rotary hoeing for controlling weeds. To reach maximum effectiveness, the hoeing must be done before the weeds reach a height of more than ¼ inch. "Timely" rotary hoeing in soybeans planted in solid stands reduced weeds and increased yields of soybeans. However, prolonged rainy periods often delay the use of the rotary hoe in farmer's fields beyond the time when it is effective. If rotary hoeing is delayed the weeds develop extensive root systems, preventing their removal with this implement.

It was previously thought that rotary hoeing should be done when the soil was dry, but in these experiments it was shown that rotary

hoeing when the soil was so wet that mud would ball up slightly on the rotary hoe was effective. Hoeings under "wet" conditions were not harmful to the soybeans and allowed rotary hoeing to be done when the weeds were at the proper stage of growth. The results from these experiments are not greatly different from those reported by Lovely et al. (3). Yields from plots receiving rotary hoeings when the soil was "wet" did not differ significantly from those applied to dry soil.

Rotary hoeing continued until the soybeans were 8 inches tall reduced the yield of soybeans, probably because of injury to the soybeans from the rotary hoe. This injury to the soybeans may have allowed weeds to get ahead of the soybeans.

The sodium salt of PCP at 20 lb/A gave excellent weed control. There was little difference between yields of soybeans from this treatment and yields from the hand-weeded plots. Shovel cultivations supplemental to PCP were adequate and necessary in most years. Rotary hoeing was of little value where PCP was used for control of weeds. Conversely, weed control resulting from pre-emergence herbicides was not significantly reduced by use of the rotary hoe, although there appeared to be more crabgrass in plots of CDAA that were rotary hoed than those not rotary hoed. CIPC at 8 lb/A and DNBP at 7½ lb/A stunted the soybeans or reduced stands and yields in some years even though weed control was good.

Hand-weeded plots gave an indication of how much increase in soybean yields can be expected if plots are kept nearly free of weeds. Perhaps this information may serve to answer the question of how much a farmer can afford to spend on chemicals to keep weeds out of soybean rows. The best jobs of hand-weeding were done in 1956 and 1957 and show that by hand-weeding the soybean yields were increased an average of 7.5 bu/A on plots cultivated twice and 4.5 bu/A on plots cultivated three times. Since farmers in the Midwest generally cultivate two or three times, these figures indicate about the maximum return that could be expected from using an ideal herbicide that would completely eliminate weeds. These yields increases may be somewhat conservative under exceptionally weedy conditions.

These experiments were conducted each year in adjoining areas and soybeans planted in rows gave higher yields than soybeans planted in solid stands.

SUMMARY

A 3-year study was conducted in soybeans planted in solid stands and in 40-inch rows to determine the effects of the rotary hoeing under "wet" and dry conditions as well as timely and late hoeing. Pre-emergence herbicides and shovel cultivations were also used in combinations with rotary hoeing.

Rotary hoeing was only moderately effective in controlling weeds but was most effective when weeds were very small. There was no difference in effectiveness when the soil was either "wet" or dry. Successful rotary hoeings were accomplished as soon as the soil was

dry enough to hold the tractor and dry enough so that mud would not ball up excessively on the rotary hoe. When good weed control was obtained with pre-emergence herbicides, rotary hoeing was not necessary.

Sodium pentachlorophenate (PCP) applied pre-emergence at 20 lb/A was outstanding in controlling weeds and increasing yields of soybeans over plots given cultivation treatments only. Yield increases with PCP averaged 4.1 bu/A more than the check when soybeans were planted in rows and 7.1 bu/A more than the check in the soybeans planted in solid stands. Injury to stands of soybeans from PCP occurred only where soybeans were planted shallow with a grain drill.

CIPC and DNBP produced severe injury to soybeans and the use of these herbicides was discontinued in these experiments. CDAA produced no apparent injury to soybeans, but did not control weeds satisfactorily.

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Combined Use of Pre-emergence Herbicides and Cross-Cultivation in Cotton

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COTTON is the major cash crop in Missouri's seven southeastern delta counties, and is second in cash value for the entire state, even though it comprises only about 6 per cent of the total acreage.

Heavy infestations of weeds late in the season materially complicate the defoliation process, reduce the efficiency of both mechanical and hand pickers, lower the grade of the lint by contributing stains and trash, and account for greatly diminished net returns. Despite advances in this field, weed control still constitutes the principal remaining barrier to complete mechanization of cotton production.

Numerous articles in the literature bear witness of the merits of both pre-emergence chemicals (1, 2, 3, 6, 7) and cross-plowing (4, 5, 8) for control of weeds in cotton. Pre-emergence herbicides have performed satisfactorily in field tests in Missouri, consistently reducing total weed control costs (including chemicals) by approximately $3.00–$7.00 per acre. Although promising, cross-cultivation has been studied less extensively. In preliminary tests cotton checked at 40-in intervals, simulating cross-plowing, received normal cross-cultivation and required less than $4.00/A for hoeing.[2]

Later experiments, including the work reported herein, were designed to study the practicality of combining the use of pre-emergence herbicides and cross-cultivation.

EXPERIMENTAL PROCEDURE

Because crabgrass (*Digitaria sanguinalis* (L.) Scop.) was a major problem, the herbicide selected for these studies was isopropyl *N*–(3–chlorophenyl)carbamate (CIPC). The tests were conducted on moderately fertile Lintonia sandy loam. The experiment was arranged in split-plot design, and each treatment was replicated six times. CIPC versus no CIPC (main plots) and cross-plowing versus drilling and thinning (sub-plots) constituted the variables. Sub-plots consisted of 14 rows, 40 in apart and 53½ ft long. Cotton (*Gossypium hirsutum* L., var. Deltapine 15) was drilled in 40-in rows on May 9, 1953. Simultaneously, CIPC[3] was applied in 12-inch bands over the rows at 6.6 lb/A in 40 gal water. A relatively high seeding rate was employed in order to obtain dense stands of cotton and the necessary thinning accounted for a part of the cost in non-cultivated plots.

All plots were cultivated the same number of times. Differences in weed growth were thus due to the direction of cultivation and/or the suppression of weeds by the chemical.

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[2]Unpublished data of the Missouri Agr. Exp. Sta., 1952.

[3]Gratuitously supplied by the Mid-South Chemical Company, Memphis, Tenn. as a 4 lb/gal emulsifiable concentrate.

The rows were barred off May 26 to 8-in bands in the drilled plots; hills were blocked off to 8-in squares in cross-plowed plots, by means of reversed disk hillers. During subsequent cultivation no soil was moved into the treated bands until the chemical lost its effectiveness. Cotton stand counts were recorded before cross-plowing, and after cross-plowing or hand-thinning (June 9).

After cross-plowing the designated plots, the entire test was hoed, and plots which were not cross-plowed were also thinned as needed. The plots were harvested on September 14 and again on October 14.

DISCUSSION OF RESULTS

Soil moisture conditions were favorable for activating the CIPC, and at the first cultivation, crabgrass was abundant everywhere except in the CIPC-treated bands. This effect was still obvious after cross plowing (Figure 1).

Whether CIPC-treated or not, the initial stand was about 52,320 plants per acre before cultural operations. After cross-cultivation, there were about 9,940 plants per acre with 18 per cent missing hills; after hand-thinning about 26,160 plants per acre. The stand resulting from cross-cultivation was considered to be well below optimum. This accentuates the need for thick uniform stands of cotton where cross-plowing is to be practiced.

The data concerning weed control, yield and earliness, and costs are summarized in the following paragraphs.

The time required for hoeing all CIPC-treated plots (3.68 hr/A) was significantly less than for all untreated plots (17.36 hr/A). Also time required for hoeing all cross-plowed plots (5.26 hr/A) was significantly less than for plots which were drilled and hand-thinned (15.61 hr/A). Furthermore, as might be expected in an experiment of this kind, there existed a high interaction between chemical and cultivation practice. This fact was confirmed statistically by comparing ratios within the tabular data. This indicates that there was a cumulative advantage to using CIPC plus cross-plowing, above the sum of their separate advantages. Expressed another way, the use of cross-plowing caused the CIPC to show up more effectively, and vice versa.

Weed control cost data reflected this same pattern. The combination practices were ranked in the following order of weed control cost per acre: (1) CIPC plus cross-plowing—$4.37, (2) cross-plowing, no CIPC—$4.65, (3) CIPC, drilling and thinning—$6.82, (4) No CIPC, drilling and thinning—$12.72. These figures include the cost of hoeing computed at $0.50/hr and the price of CIPC computed at $7.50/gal. Assuming that the spray equipment is already owned, no additional costs need be considered for accurate comparison, since CIPC was applied at planting at no extra cost, the entire test received the same number of cultivations, and all plots were planted originally at the same rate of seed per acre, thus making seed costs equal. The slight increase in man hours required for mounting, filling, calibrating and cleaning the sprayer may be ignored for all practical purposes.

Figure 1. Hills of cotton formed by cross-cultivation with reversed disk hillers 4 weeks after planting. Note abundant growth of crabgrass in untreated check (above) and excellent weed control due to 6.6 lb CIPC per acre (below).

Neither earliness (indicated by per cent yield at first picking) nor total yield was affected by the use of CIPC. Total yields, which ranged around 1750 lb/A seed cotton, were not significantly different between all cross-plowed plots and all drilled and thinned plots, however, cross-plowed plots were significantly later maturing. Only about 42 per cent of the total yield was harvested at first picking in

the cross-cultivated plots, in comparison with more than 60 per cent in drilled and thinned plots. These results can probably be explained by the fact that the growth and feeding range of cotton are adjustable over quite a wide range of spacings without materially affecting the yield. Sparse, rank cotton is commonly delayed in maturity in comparison with denser plant populations.

In addition to reducing the man hours required for thinning and for weed control, both the use of pre-emergence chemicals and cross-plowing reduce the critical need for timeliness in hoeing during the interval between planting and post-emergence oiling or flame-cultivation.

Disadvantages of cross-plowing, however, include an increase in pounds of seed cotton required for planting, a significant reduction in mechanical picker efficiency (7), and the possibility that cross-plowed cotton may be later maturing. Especially under conditions of heavy moisture and/or high nitrogen fertilization, cross-plowed cotton has a tendency to produce excessive vegetative growth and to lodge, thereby rendering harvesting more difficult.

SUMMARY

1. Combinations of cross-cultivation and CIPC as a pre-emergence spray were evaluated for weed control and effect upon cotton.

2. The results indicate that the two practices may be used in combination both effectively and economically.

3. There were no deleterious effects upon the cotton, except that thinly spaced, cross-plowed cotton was significantly later maturing. Under Missouri's short growing season, retardation of maturity must be considered an objectionable feature which is only slightly mitigated by seeding at excessive rates. The results emphasize the necessity for thick uniform stands of cotton to insure against poor stands and missing hills if cross-plowing is to be practiced.

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Effectiveness of Aerial Application of Herbicides for Hardwood Control in Louisiana

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INTRODUCTION

THE control of undesirable woody plants is one of the biggest land management problems on non-crop land in the South. There are more than six million acres of pine sites in Louisiana alone that are in need of cull hardwood control (12). Large acreages of similar stands and sites are located in other southern states. Because of the competition for land and increased demand for raw materials, many landowners have realized the necessity of utilizing their land to the fullest extent.

In general, the low-volatile esters of 2,4,5–trichlorophenoxyacetic acid (2,4,5–T) and 2–(2,4,5–trichlorophenoxy)propionic acid (silvex) are effective on many species commonly encountered in upland hardwood areas when applied as aerial sprays. An ester of 2,4,5–T has proved to be better than esters of 2,4–D and mixtures of esters of 2,4–D and 2,4,5–T for control of blackjack and post oaks (2, 4, 5). Also, esters of 2,4,5–T and silvex are more selective on pines and other conifers than 2,4–D (1, 2, 4). Two pounds per acre of a low-volatile ester of 2,4,5–T applied as an aerial spray has not caused excessive damage to pine seedlings one year old or older (2, 4, 6, 9, 11). Most research workers agree that application of the herbicide in a 1:3 ratio of oil and water is suitable for aerial application, because the larger droplet size of emulsions compared with oil alone reduces drift hazards (3, 4, 5, 8). The use of low-volatile esters of these phenoxy herbicides reduces hazard to crop plants (2, 4).

Foliage sprays have been found to be most effective from about May 15 to July 1 in the South (2, 4, 5, 8, 10). For best results, the herbicides need to be absorbed and translocated through the stems and to the roots of the hardwoods. This translocation seems to be closely associated with organic foods (3). The rate of translocation away from the point of application is generally slow. The herbicides move through the phloem tissue of the plant, and if the concentration in the carrier is too strong the plant tissue may be killed at the point of application before it can be absorbed and transmitted to the stem and roots (2, 3).

Hardwoods were killed in Louisiana with 2 pounds per acre of propylene glycol butyl ether esters of 2,4,5–T in an oil-water emulsion applied at 5 gallons per acre during May. Treatments in September 1955 and in April 1956 gave insufficient kill of hardwoods for good growth of pines (2). Good control of pole-size maple and oak has been obtained in the Lake States from the application of 1 pound

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per acre of an ester of 2,4,5–T (1). Single applications did not give desirable control in Oklahoma and additional control was obtained with retreatments (5, 8).

Aerial spraying is advantageous in that low volumes can be applied uniformly over large areas, and large hardwoods are treated along with low brush. Disadvantages include possible drift of herbicides to sensitive cultivated plants, failure to reach all understory vegetation, and failure to kill some species. The season for aerial application of herbicides for woody plant control usually coincides with the time sensitive crop plants are being grown. State regulations regarding use of these materials must be observed.

METHODS AND MATERIALS

The experiments reported here were conducted on cutover forest land in southwestern Louisiana. The purpose was to study the effects of 7 herbicidal treatments for control of undesirable woody plants when applied as aerial sprays. Treatments were applied on May 30, 1957 between 6:15 a.m. and 9:15 a.m., the wind velocity being around 2 miles per hour. All treatments were replicated twice on plots 200 feet wide and 1,320 feet long, with 100-foot buffer strips between plots. There was no rainfall for more than 3 days after spraying. All spray materials were applied in a volume of 5 gallons per acre by a Stearman biplane with gravity-flow spray equipment (Figure 1). The herbicides were applied on swath centers spaced 20 feet apart.

The treatments included the amine salt of 2–(2,4–dichlorophe-

Figure 1. Aerial application of herbicides for woody plant control in western Louisiana.

noxy)–propionic acid [2–(2,4–DP)] applied at 1 pound per acre, the amine salt of silvex applied at 1, 2, and 3 pounds per acre, an amine salt and the butoxy ethanol ester of 2,4,5–T applied at 2 pounds per acre, and the propylene glycol butyl ether ester of silvex applied at 2 pounds per acre.[3] The amine formulations were applied in water, and the ester formulations were applied in a water-oil mixture (3.5 parts water and 1.5 parts diesel oil and herbicide).

The timber type in the experimental area was mixed hardwoods with a small number of pines per acre and the size of all species varied from small seedlings to sawlog size. Longleaf *(Pinus palustris)*, loblolly *(P. taeda)*, and shortleaf *(P. echinata)* pines were present. The major hardwood species present were: post oak *(Q. stellata)*, blackjack oak *(Q. marilandica)*, red oak *(Q. falcata)*, willow oak *(Q. phellos)*, sweetgum *(Liquidambar styraciflua)*, blackgum *(Nyssa sylvatica)*, and hickory *(Carya tomentosa)*. Other species present were: ash *(Fraxinus americana)*, dwarf sumac *(Rhus copallina)*, red maple *(Acer rubrum)*, white oak *(Q. alba)*, cherry *(Prunus serotina)*, black oak *(Q. velutina)*, dogwood *(Cornus florida)*, water oak *(Q. nigra)*, and several other minor species.

At the time of spraying, live trees were grouped into 4 size classes along a transect line, averaging about 10 feet in width, through the center of each plot. The transect lines terminated 2 chains from each of the plot ends. The tree size classes used were: under 1 inch in diameter, 1 to 4 inches, 4 to 10 inches, and over 10 inches. Numbered aluminum tags were used for tagging trees, and the tagged trees were marked with tree-marking paint.

Soil moisture at the time of spraying was adequate for rapid growth, and rainfall was near normal during the 1957 and 1958 growing seasons.

The effects of the herbicides were recorded in July 1958, 14 months after the spraying. The percentage of top-kill was estimated visually for each tagged tree, and the numbers and heights of basal sprouts were recorded. Also, crown-class information was recorded to indicate whether each tree was dominant, co-dominant, intermediate, or overtopped.

RESULTS AND DISCUSSION

The data are summarized in Table 1. Percentages of kill are shown separately by species because of the great variability in kill. The average kill shown for each treatment is an approximate measure of the relative effectiveness of the treatment in releasing growing space for desirable plants. However, the treatment averages are not strictly comparable, because all species did not occur on all plots.

The most effective treatment was the butoxyethanol ester of 2,4,5–T, 2 pounds per acre, with an average top-kill of 70 percent (Figure 2). Better top-kills were recorded for sweetgum, post oak, and blackjack oak than were recorded for black oak, red oak, blackgum, white

[3]The authors wish to acknowledge the cooperation of the Anchem Products Company and the Dow Chemical Company in supplying the compounds for this experiment.

Table 1. Top-kill of deciduous species in four diameter classes produced by herbicides applied 14 months previously.

Species and size	Pounds of herbicide applied per acre in 5 gallons of carrier[a]						
	2 lb. 2,4,5-T ester	2 lb. silvex ester	2 lb. 2,4,5-T amine	2 lb. silvex amine	3 lb. silvex amine	1 lb. silvex amine	1 lb. 2-(2,4-DP) amine
	Average percent top-kill						
Blackjack oak							
Under 1"	93	100	100	60	39	69	98
1"–4"	93	100	39	24	66	62	18
4"–10"	96	99	39	39	52	77	12
Over 10"	—	—	—	—	41	—	31
Average	94	99	59	41	50	69	40
Post oak							
Under 1"	95	99	37	100	71	59	15
1"–4"	100	100	35	11	99	48	19
4"–10"	100	88	55	41	47	43	8
Over 10"	94	100	89	32	74	43	10
Average	98	97	54	46	73	48	13
Red Oak							
Under 1"	50	83	8	16	38	6	4
1"–4"	70	70	14	9	71	12	3
4"–10"	95	77	17	6	52	9	3
Over 10"	100	71	25	7	22	3	3
Average	79	75	16	10	46	7	4
Sweetgum							
Under 1"	94	83	37	42	68	23	4
1"–4"	100	41	56	11	7	21	3
4"–10"	100	41	91	—	16	9	3
Over 10"	100	49	71	3	75	—	—
Average	99	53	64	19	42	18	4
Blackgum							
Under 1"	100	81	78	65	87	21	18
1"–4"	81	100	100	5	66	—	3
4"–10"	—	21	81	3	52	—	3
Over 10"	41	—	—	—	—	6	3
Average	74	67	86	24	68	13	7
Hickory							
Under 1"	93	76	9	3	—	3	3
1"–4"	77	65	7	3	11	3	3
4"–10"	—	100	22	3	18	3	3
Over 10"	21	53	25	3	—	3	3
Average	64	74	16	3	14	3	3
Red maple							
Under 1"	13	10	7	3	3	3	3
1"–4"	11	—	—	—	—	3	—
4"–10"	—	7	—	3	11	5	3
Over 10"	—	—	—	—	—	—	—
Average	12	8	7	3	7	4	3
White oak							
Under 1"	51	—	3	3	11	18	—
1"–4"	100	16	3	—	46	—	—
4"–10"	31	55	—	3	44	—	—
Over 10"	100	82	11	7	10	—	3
Average	71	51	6	4	28	18	3
Water oak							
Under 1"	28	12	—	5	36	—	3
1"–4"	—	—	3	—	81	—	—
4"–10"	—	51	11	3	11	—	3
Over 10"	—	34	4	—	3	—	3
Average	28	32	6	4	33	—	3
Black oak							
Under 1"	67	21	—	11	—	—	—
1"–4"	91	61	—	11	—	6	—
4"–10"	100	—	—	11	46	3	—
Over 10"	—	—	—	31	11	—	—
Average	86	41	—	16	28	4	—
Average all species	70	60	35	25	39	21	9

[a]Ester formulations were applied in 1.5 gallons of oil and 3.5 gallons of water per acre, and amine formulations were mixed with water and applied at 5 gallons per acre.

Figure 2. Appearance of a mixed hardwood stand 16 months after treating with 2 pounds per acre of 2,4,5–T ester applied as an aerial spray.

oak, and hickory even though fair to good kills were obtained for these species. Poor top-kills were obtained on water oak and red maple. The treatment second in effectiveness was the propylene glycol butyl ether esters of silvex, 2 pounds per acre. Top-kill for all species averaged 60 percent. The best top-kills were recorded for blackjack oak and post oak. However, good top-kills were obtained on red oak, hickory, and blackgum. The percentages of average top-kill were less than which is desirable for sweetgum, white oak, and black oak. Water oak and red maple were only slightly affected. These two treatments provided effective release of growing space for pines and forage plants.

The amine salts of 2,4,5–T, silvex, and 2–(2,4–DP) were considerably less effective than the esters of 2,4,5–T and silvex. These treatments are not considered generally satisfactory for killing hardwoods, although some species were severely damaged by them.

In general, the herbicides tested were more effective on stands on the ridges and slopes than those in creek bottoms. However, this seemed to be due to a difference in species growing in each location. Species such as white oak, water oak, hickory, blackgum, and red maple, which are most often found in creek bottoms, were less susceptible to herbicide treatments than blackjack oak, post oak, and

red oak, which usually make up the major part of the stand on the ridges and slopes of the cut-over longleaf pine area of Louisiana and east Texas.

Top-kill was affected somewhat by size of stems, but in some instances an herbicide killed certain species consistently regardless of size.

Based on the results obtained thus far, a low-volatile ester of 2,4,5–T appears to be the best herbicide for the control of upland hardwoods in Louisiana. It is more effective on a wider range of species encountered in southwest Louisiana than an ester of silvex. The species controlled effectively by an ester of silvex seem to be controlled equally as well by 2,4,5–T, and at present the latter costs slightly less. Basal sprouting was negligible from all species treated with these two herbicides. Top-kill was not greatly affected for these two herbicides for species growing on ridges and slopes by the position of the tree crowns in the stand, whether dominant, co-dominant, intermediate, or overtopped. In multi-storied stands, the herbicides fail to reach understory brush in sufficient quantity to be effective.

The herbicides had relatively little effect on longleaf and loblolly pines following spray applications. However, there was some abnormal shedding of needles and burning of some of the new terminal growth of exposed loblolly pines. Most of this spray damage disappeared within a year after spraying.

SUMMARY

A test of 7 herbicidal treatments for controlling undesirable hardwood trees was made in May 1957 in southwestern Louisiana. Herbicidal solutions were sprayed by airplane at the rate of 5 gallons per acre. Results 14 months later indicated that the most effective treatment for hardwood control was the butoxyethanol ester of 2,4,5–T, applied in oil-water emulsion at the rate of 2 pounds per acre. This treatment gave effective control of blackjack oak, post oak, black oak, and sweetgum; partial to good kill of red oak and blackgum; partial kill on hickory and white oak; and in most instances red maple and water oak were not severely damaged.

The propylene glycol butyl ether ester of silvex, applied in oil-water emulsion at 2 pounds per acre, ranked second in effectiveness. It was as effective as the ester of 2,4,5–T on blackjack oak, post oak, red oak, and hickory but less effective on sweetgum, blackgum, white oak, and black oak.

The amine salt formulations of 2,4,5–T, silvex, and 2–(2,4–DP) were much less effective than the ester formulations.

The herbicides effected better kills on ridges and slopes than in the creek bottoms, because the more susceptible species are commonly found on ridges and the more resistant species are common in bottoms. Basal sprouting was negligible. Good kills were obtained of susceptible species that commonly grow on the ridges and slopes regardless of size or crown class. Herbicide damage to pines was negligible.

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Herbicidal Activities of Halogenoalkylcarboxylic Acid Esters I. Germination Inhibition Activities[1]

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INTRODUCTION

HALOGENOALKYLCARBOXYLIC acids such as monochlorocetic acid, trichloroacetic acid, dichloropropionic acid and 2,3–dichloroisobutyric acid, have well known herbicidal activities upon monocot plants. However, they are not effective in killing the seeds of weeds. This may be due to their inability to penetrate the seed coats.

In a search for a compound that would not only penetrate readily but also retain its herbicidal activity, the esters of halogenoalkylcarboxylic acids were synthesized and tested. P. Poignant investigated the herbicidal activities of monochloroacetic acid esters using rutabaga (3). In the present investigation forty-six esters of this series have been synthesized in this laboratory and some of them are new compounds. A preliminary evaluation is reported on the ability of these chemicals to prevent the germination of seed of rape (Brassica campestris L.), millet (Echinochloa crusgalli (L.) Beauv. var. frumentacea (Roxb.) W. F. Wight) and rice (Oryza sativa L.).

MATERIALS AND METHODS

The esters were prepared by the reaction of alcohols or phenols with the appropriate acids, and with sulfuric acid or hydrogen chloride gas for a catalyst. In other cases alcohols or phenols were reacted with appropriate acid chlorides in pyridine. The vinyl esters were prepared by the reaction of acid and acetylene gas according to the method of Wiley (4). Details of the methods of preparation and chemical properties of the compounds will be reported elsewhere.

The germination inhibition test was as follows: Three milliliters of the appropriate concentration of emulsion or solution was introduced into a petri-dish containing a filter paper on the bottom. Occasionally a small amount of distilled water was added later to maintain the moisture content in the dish. Each dish contained fifty seeds which were placed upon the filter paper and allowed to germinate at 25° C constant temperature. Absolute dark conditions were not adhered to since light was used during each inspection. The rate of germination was checked at 24, 48 and 72 hours. Although the petri-dish cover was used, the dishes were not sealed airtight. The test for each chemical was repeated several times to check

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for reproducibility. The seed-killing rating scale of Hamm and Speziale (1, 2) was used in these experiments, as follows:

Percent germination	Seed-killing rating
0–25	3
26–50	2
51–75	1
76–100	0

The seed-killing effect was rated in each of three trials and the sum taken. The sum was then rated according to the table below. This rating scale was arbitrarily determined to follow the scale previously set (1, 2).

Summation of individual seed-killing ratings	Summation, seed-killing rating
9	4
7–8	3
5–6	2
3–4	1
1–2	0

RESULTS

The results are shown in Table 1. The concentrations used during the test were 0.1 and 0.05% (w/v). When a compound showed complete inhibition at the above concentrations a lower concentration was tried to determine the limit of effectiveness. In a few cases penetration through the seed coat was insufficient and initial germination occurred. However, immediately upon germination the contact with the chemical compound killed the seedling. These cases were rated as killed and included in the table. They were not listed in a separate category.

DISCUSSION

In general, most of the esters listed in Table 1 showed stronger seed-killing activities than the respective halogenoalkylcarboxylic acid sodium salts. In regard to the alkyl moiety of esters, the allyl and butoxyethyl in the aliphatic groups showed stronger activities than the others. In the aromatic groups the benzyl, phenylethyl and substituted phenyl radicals showed striking seed-killing activities. The activities of the esters of chloroacetic acid, dichloropropionic acid, trichloroacetic acid and dichloroisobutyric acid were in that general order, with chloroacetic acid much more active. In the monohalogen substituted acetic acid esters the substitution of bromine and iodine showed stronger activities than chlorine.

Increasing the length of the carbon chain in the acid did not increase the activity. With substitution of phenyl radicals in esters, halogen, methyl and nitro groups contributed to higher activities, but the number and position of chlorine substitutions in the phenyl radical did not affect the activities.

As these esters are rather volatile in general, the residual effect for young rice plants transplanted from seedling beds may be assumed

Table 1. Seed-killing activity of halogenoalkylcarboxylic acid esters.

Chemicals	Rape		Millet		Rice		Sum		Rating	
	A[a]	B[b]	A	B	A	B	A	B	A	B
Chloroacetates										
Sodium	0	0	0	0	0	0	0	0	0	0
Methyl	0	0	0	0	3	0	3	0	1	0
Ethyl	0	0	0	0	3	0	3	0	1	0
Propyl	0	0	0	0	2	0	2	0	0	0
Isopropyl	0	0	3	0	2	0	5	0	2	0
Butyl	0	0	1	0	0	0	1	0	0	0
Isobutyl	0	0	0	0	1	0	1	0	0	0
sec-Butyl	0	0	0	0	0	0	0	0	0	0
Amyl	0	0	0	0	1	0	1	0	0	0
Vinyl	0	0	1	0	2	0	3	0	1	0
Allyl	3	1	3	3	3	1	9	5	4	1
Methoxyethyl	3	2	0	0	0	0	3	2	1	0
Butoxyethyl	3	3	3	0	3	0	9	3	4	1
Furfuryl	3	0	0	0	1	0	4	0	1	0
Tetrahydrofurfuryl	3	0	1	0	2	0	6	0	2	0
Benzyl	3	3	1	0	3	0	7	3	3	1
Phenylethyl	3	3	3	0	3	0	9	3	4	1
p-Methoxyphenylethyl	3	3	3	1	3	2	9	6	4	2
o-Chlorophenyl	3	1	3	3	3	1	9	5	4	2
m-Chlorophenyl	3	3	3	3	3	3	9	9	4	4
p-Chlorophenyl	3	3	3	3	3	3	9	9	4	4
2,4-Dichlorophenyl	3	3	3	2	3	2	9	7	4	3
2,4,5-Trichlorophenyl	3	3	3	2	3	1	9	6	4	2
2,4,6-Trichlorophenyl	3	3	3	3	3	3	9	9	4	4
Pentachlorophenyl	3	3	3	3	3	3	9	9	4	4
p-Bromophenyl	3	3	3	3	3	0	9	6	4	2
p-Iodophenyl	3	1	3	3	3	0	9	4	4	1
p-Nitrophenyl	3	3	3	3	3	3	9	9	4	4
p-Methylphenyl	3	3	3	3	3	0	9	6	4	2
Trichloroacetates										
Sodium	0	0	0	0	0	0	0	0	0	0
Allyl	3	0	2	2	3	2	8	4	3	1
Butoxyethyl	0	0	0	0	0	0	0	0	0	0
Tetrahydrofurfuryl	0	0	0	0	0	0	0	0	0	0
Benzyl	3	1	0	0	0	0	3	1	1	0
Dichloropropionates										
Sodium	0	0	3	0	0	0	3	0	1	0
Allyl	0	0	2	0	3	0	5	0	2	0
Butyl	0	0	0	0	0	0	0	0	0	0
Butoxyethyl	3	3	0	0	3	2	6	5	2	2
Benzyl	3	3	0	0	0	0	3	3	1	1
2,3-Dichloroisobutyrates										
Sodium	0	0	3	0	0	0	3	0	1	0
Vinyl	0	0	0	0	0	0	0	0	0	0
Butoxyethyl	0	0	0	0	0	0	0	0	0	0
Benzyl	3	2	0	0	0	0	3	2	1	0
Allyl	0	0	0	0	0	0	0	0	0	0
Bromoacetates										
Sodium	0	0	0	0	0	0	0	0	0	0
Allyl	3	3	3	3	3	2	9	8	4	3
Butoxyethyl	3	3	3	3	3	1	9	7	4	3
Benzyl	3	3	3	0	3	0	9	3	4	1
Iodoacetates										
Sodium	0	0	0	0	0	0	0	0	0	0
Allyl	3	3	3	3	3	3	9	9	4	4
Butoxyethyl	3	3	3	3	3	1	9	7	4	3
Benzyl	3	3	3	3	3	1	9	7	4	3

[a]Concentration 0.1 per cent (w/v).
[b]Concentration 0.05 per cent (w/v).

to be negligible. However, herbicidal tests in greenhouses and fields will be carried out to determine the extent of residual toxicity for rice seedlings as well as seed-killing potency under conditions of economic use.

SUMMARY

A comprehensive series of halogenoalkylcarboxylic acid esters, including several new compounds, were synthesized. Their inhibitory effects on the germination of rape, millet and rice seeds were tested and observed. In general the esters showed greater germination inhibition than their respective parent acids. Greater activity was shown by those compounds possessing allyl, butoxyethyl, benzyl and substituted phenyl as the alcohol moiety of the esters. The kind of halogen in halogenoalkylcarboxylic acid influenced the inhibition. Esters of bromoacetic and iodoacetic acids were strikingly more effective than corresponding esters of chloroacetic acid. Increase in the number of chlorine substitutions on the acid moiety decreased the inhibitory action.

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Wild Garlic Bulblet Viability Determined by 2,3,5–Triphenyltetrazolium Chloride[1]

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WILD garlic (*Allium vineale* L.) continues to be an important problem weed of Eastern and Southeastern United States. It is spread principally by aerial bulblets mixed with seed. The present rules and regulations used by seed laboratories to determine bulblet viability seem inadequate. This paper will discuss the use of 2,3,5–triphenyltetrazolium chloride, referred to as TZ, as an indicator of bulblet viability.

Methods of reproduction of the garlic plant were well understood as early as 1914 (1). Control is made difficult by its means of propagation: (a) aerial bulblets which germinate in the fall, (b) soft-shelled below-ground bulbs which germinate in the early fall, (c) hard-shelled below-ground bulbs which germinate in late fall and spring, but may remain dormant for two seasons or more, and (d) small wrinkled black seeds, the germination requirements of which are not well known (2) (Figure 1). Consideration will be given to only the aerial bulblets in this paper.

Figure 1. Wild garlic reproduces by seed, aerial bulblets, soft-shelled bulbs, and hard-shelled bulbs.

Aerial bulblets are harvested with wheat, oats, barley, ryegrass, orchard grass, fescue, and with the seed of many other crops harvested during the summer. The movement of seeds of these crops is an important method of spreading these bulblets.

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U.S.D.A. Rules and Regulations Under the Federal Seed Act (5) describe inert matter in detail. According to these rules and regulations certain wild onion bulblets and wild garlic bulblets are considered as inert matter (and therefore dead). Such bulblets are defined as, "Bulblets of wild onion and wild garlic *(Allium)* which are devoid of the husk and pass through a 10 x 10 mesh screen (10 mesh per inch) made of 26 gage (0.020 inch diameter) stainless steel wire; bulblets which are devoid of the husks and are retained on 10 x 10 mesh screen, but which show injury to the basal end; bulblets which show evident damage to the basal end and have part of the husk removed;. . .". In addition to the above regulation, the following interpretation is used by seed laboratories. "All bulblets which are devoid of husk and which pass through the screen are to be considered inert matter. Those which do not pass through, shall be considered good only when the basal end is not damaged. All bulblets which have part of the husk removed shall be considered good if there is no evident damage to the basal end."[3] Estimates of viability are thus determined on the basis of size, presence of husks, and external evidence of injury to the basal end. Viability is thus not directly determined by the evidence of life within essential bulblet structures.

The time required for growth tests on germination pads, in petri dishes, or in soil, plus the irregularities resulting from bulblet dormancy, greatly restrict the usfulness of these tests in a law enforcement program. The existence of these barriers probably accounts for the absence of reliable viability tests.

Rapid color tests for seed viability are now available which should provide a renewed challenge to seed analysts concerned with more accurate germination determinations for wild garlic. One of the most promising chemicals that can be used is 2,3,5–triphenyl tetrazolium chloride (3, 4). This chemical is colorless when dissolved in water, but when in contact with living cells it is enzymatically reduced to an intensely reddish colored insoluble formazan. This precipitates at the sites of enzymatic activity. Consequently, living tissue is stained red, and dead tissue does not change in color. Viability is determined by observation of the stain pattern of vital structures. Vigor of the bulblet is indicated by the rapidity and intensity of color change. The entire test can be made in less than 48 hours, including the bulblet moistening period.

METHODS AND MATERIALS

1952 Studies.

Seed of tall fescue heavily infested with garlic bulblets was obtained from the N. C. State Seed Laboratory in the fall of 1952. The seed had been submitted for routine testing.

Bulblets were removed from the fescue seed by screening and hand picking. The bulblets were hand separated into two groups accord-

[3]Mimeographed "Rules for Testing Seeds Adopted by Association of Official Seed Analysts Effective July 1, 1955. Federal Seed Act Regulations amendments effective Nov. 19, 1955."

ing to size, one small and the other large. Eight sub-samples of 50 bulblets each were obtained from each size group. The weight of each sample was determined. Four small bulblet samples and four of the large bulblet samples were planted ½ inch deep in a flat of heat-sterilized Norfolk sandy loam on October 11, 1952. The flat was then subjected to outdoor temperatures. The number of garlic seedlings, and their height was determined on November 11, 1952 (Table 1).

Table 1. Wild garlic bulblets germinated and grown in soil, compared to germination color test using 2,3,5–triphenyl tetrazolium chloride (TZ). Average of 4 replications with 50 bulblets each. 1952 experiment.

Bulblet size	Color tests using TZ reagent		Germinated and grown in soil		
	Weight of 50 bulblets, mg.	Viable percent	Weight of 50 bulblets, mg.	Germination percent	Av. height, mm., one month after planting
Large	1135	79	1227	79	77
Small	620	74	603	76	55
Germination percentages			L.S.D.	N.S.	
Weight of bulblets used in color test compared to those grown in soil. Large compared with large, and small with small			L.S.D.	N.S.	
Height of plants grown from large bulblets compared to those grown from small bulblets			L.S.D. (.05) (.01) C.V.	4.8 mm. 7.2 mm. 4%	

Bulblets from each of the remaining four sub-samples of large and small bulblets were placed individually on moistened filter paper in petri dishes. After 36 hours the bulblets were cut in half, longitudinally, with a razor blade. One-half of each bulblet was placed in a 1% aqueous solution of 2,3,5–triphenyl tetrazolium chloride. Apparent viability, used in this paper to indicate germination, was determined after 1 hour of staining. Those halves showing a red color in both the stem and root producing area were considered as viable.

1957 Studies.

A second study was started during the summer of 1957. Bulblets were hand collected from fields on July 15, 1957 and stored in paper bags in the laboratory at room temperature. The samples were prepared for planting on August 21–28.

Bulblets that were too large to pass through an $^{8}/_{64}$ inch round-hole screen were discarded. Those that did pass through were later separated into large and small sizes by the use of a 10 x 10 mesh screen, the size referred to in the Federal Seed Act Regulations (5). Subsamples of both large and small bulblets were prepared as follows:

(1) Sound bulblets—bulblets free from cracks or other visible damage.
(2) Cracked bulblets—each bulblet squeezed with tweezers to crack the hull. Basal root area was not damaged.
(3) Devoid of husks—hull torn free by the use of tweezers.

Each of the three groups was prepared from bulblets selected at random. After preparation, each group of 20 bulblets was placed in a small paper coin envelope and stored in a bulk sample of wheat, to simulate normal bulblet storage in seed. The envelopes of bulblets were not removed from the wheat until the time of testing. Samples for each group were prepared (45 of each method of preparation), to provide material for 5 dates of testing in soil, petri dish germination tests, and TZ germination tests (Table 2). The five dates of testing were September 1, 1957, October 1, 1957, November 1, 1957, January 1, 1958, and March 1, 1958. Three replications were used.

Seedlings that emerged in the soil tests were counted at intervals over a 7 month period. Only those bulblets that germinated and produced above-ground plants were counted. No attempt was made to determine if some bulblets were dormant and still viable in the soil at the end of the 7 month period.

The bulblets that germinated on moistened filter paper in petri dishes were counted at intervals over a 9 month period. Seedlings were removed from the test as counted. Those that softened due to rot were removed and discarded.

The TZ germination test was conducted as previously discussed.

RESULTS AND DISCUSSION

1952 Studies.

Using bulblets separated from commercial fescue seed, it is evident that the garlic bulblet viability was retained at relatively high percentages, with the average ranging from 74 to 79 per cent (Table 1). There were no statistical differences in percentage germination between the large bulblet and small bulblet groups. The TZ test indicated essentially the same percentage germination as that obtained at the end of one month's growth in heat-sterilized soil.

One month after planting, garlic plants grown from large bulblets were about 50 per cent taller than plants grown from small bulblets.

1957 Studies.

Germination results from the 1957 hand-harvested, specially conditioned bulblets are presented in Table 2. In addition to being prepared and separated into sound, cracked, and devoid of husks groups, each group was further divided into large and small sizes.

The bulblets devoid of husk have the shortest span of life. However, if planted immediately after removal of the husk germination may equal that of sound bulblets. Bulblets devoid of husk and stored for 2 months showed an average germination percentage of 6.8 compared to 22.5 per cent for sound bulblets. Cracked bulblets were intermediate in length of life, and the sound bulblets had the longest duration of life. Size of bulblets appeared to have no great influence on length of life. Where differences existed, usually the larger bulblets showed slightly higher germination.

In the September 1 planting, relatively high numbers of bulblets germinated in the soil test regardless of whether the bulblets were

Table 2. Average per cent of bulblets to germinate. Each figure represents the average of 3 replications, with 20 bulblets per replication. 1957 experiment.

Date planted or date of germination test		Germinated in soil	Germinated in petri dish	TZ Viability test
		per cent	per cent	per cent
September 1				
Sound bulblets	—large	88	40	92
Sound bulblets	—small	85	43	88
Cracked bulblets	—large	83	63	82
Cracked bulblets	—small	67	30	72
Devoid of husk	—large	98	70	75
Devoid of husk	—small	78	45	78
L.S.D. (.05)		7.1		
(.01)		9.5		
C.V.		14%		
October 1				
Sound bulblets	—large	75	95	92
Sound bulblets	—small	67	83	65
Cracked bulblets	—large	88	83	70
Cracked bulblets	—small	42	23	45
Devoid of husk	—large	20	27	38
Devoid of husk	—small	10	8	18
L.S.D. (.05)		6.3		
(.01)		8.5		
C.V.		17%		
November 1				
Sound bulblets	—large	30	90	78
Sound bulblets	—small	15	75	67
Cracked bulblets	—large	48	92	82
Cracked bulblets	—small	3	17	25
Devoid of husk	—large	10	17	27
Devoid of husk	—small	3	8	13
L.S.D. (.05)		6.6		
(.01)		8.8		
C.V.		24.8%		
January 1				
Sound bulblets	—large	52	92	95
Sound bulblets	—small	17	73	62
Cracked bulblets	—large	37	47	52
Cracked bulblets	—small	12	18	20
Devoid of husk	—large	3	2	8
Devoid of husk	—small	0	2	5
L.S.D. (.05)		4.4		
(.01)		5.9		
C.V.		19.7%		
March 1				
Sound bulblets	—large	0	93	97
Sound bulblets	—small	15	87	73
Cracked bulblets	—large	0	30	27
Cracked bulblets	—small	0	7	13
Devoid of husk	—large	0	0	5
Devoid of husk	—small	0	0	5
L.S.D. (.05)		3.4		
(.01)		4.5		
C.V.		19%		
For all 5 dates combined; to compare between germinated in soil, in petri dish and TZ test			L.S.D. (.05)	10.0
			(.01)	13.0
			C.V.	19%
For all treatments combined; to compare between sound bulblets, cracked bulblets, and husk removed			L.S.D. (.05)	6.1
			(.01)	8.0
			C.V.	19%
For all treatments combined; to compare large bulblets vs small bulblets			L.S.D. (.05)	2.0
			(.01)	2.7
			C.V.	19%

sound, cracked, or devoid of husk. The lowest per cent to germinate was 67 per cent. Only small differences in emergence existed between the large and small bulblet groups, with a slightly larger number of the large bulblets germinating. Viability as determined by the TZ method was reasonably close to the number that emerged from the soil.

The number of bulblets germinating in the petri dishes was lower than when planted in the soil or as determined by the TZ test. The low petri dish germination percentages probably resulted from high laboratory temperatures. The germinator was located in a laboratory on the west side of the building. This laboratory became very hot in the afternoon through the summer months, continuing through the month of September. It is believed that these high temperatures favored the development of dormancy and microbial decomposition. The excessive heat apparently did not interfere with the validity of the TZ readings.

In the October 1 planting in the soil, the sound and large cracked bulblet groups gave relatively high germination, the lowest percentage being 67. Those devoid of husk gave the lowest germination with 20 per cent of the large and 10 per cent of the small bulblet groups germinating. Even with the small number of bulblets germinating, the percentage would be of considerable importance in heavily infested seed. Results from the petri dish and the TZ tests corresponded reasonably closely to those of the soil test.

By November 1, only a small number of the bulblets planted in soil germinated. The largest percentage was 48 and the lowest 3. The cold soil temperatures were apparently unfavorable for germination and survival. When similar bulblets were germinated in petri dishes or evaluated by TZ tests, much larger numbers were observed as viable. The largest percentage was 92 and the lowest 8. The sound bulblets, both large and small, and the large cracked bulblets germinated in relatively large numbers. The small cracked bulblets and those devoid of husk, both large and small, showed low viability. The numbers were large enough, however, to be of considerable importance if seeds were planted which were heavily infested with such bulblets.

In the January 1 tests the sound bulblets were still capable of giving large numbers of viable seedlings, as indicated by the petri dish and the TZ tests. The viability of the cracked bulblets and bulblets devoid of husk was considerably reduced. The emergence of bulblets planted in the soil was considerably lower than estimates of germination by the other two methods. It is assumed that low temperature effects, which presumably reduced germination for the November 1 planting, were still in operation.

In the March 1 tests, the sound bulblets were still capable of giving large numbers of viable seedlings, as indicated by the petri dish germination test and the TZ tests. The cracked bulblets were still capable of producing enough plants to be of considerable economic importance in seed. Even some of those bulblets which were devoid of husk were capable of germinating under favorable conditions.

However, when planted in soil all except the small sound bulblets failed to germinate. It is believed that soil temperatures had increased sufficiently to induce dormancy. In addition, the weakened bulblets probably showed increased susceptibility to attack by soil microorganisms.

SUMMARY

1. Both large and small sized garlic bulblets were equally viable if stored for only a short period of time. With longer storage the larger bulblets usually showed the higher germination percentage.
2. Bulblets damaged by cracking the husk, or by complete removal of the husk, will germinate readily if planted under favorable conditions soon after the bulblet is injured. Some germination can be expected for as long as 3 months.
3. In storage, bulblets devoid of husk have the shortest duration of life, cracked bulblets were intermediate, and those that were sound had the longest duration of life.
4. Bulblets which were devoid of husk and which passed through the 10 x 10 screen gave germination percentages of 78% when planted in soil immediately after injury. Such bulblets are classed as inert matter, and thus dead, under the Federal Seed Act (5).
5. The TZ method was found to be a reliable method for determining bulblet viability, and can be performed quickly and efficiently. It is believed that this technique is superior to the method described in the U.S.D.A. Rules and Regulations under the Federal Seed Act.

ACKNOWLEDGMENT

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The Metabolism of Nut Grass (*Cyperus rotundus* L.) I. The Influence of Various Oxygen and Carbon Dioxide Levels Upon Germination and Respiration

R. D. Palmer and W. K. Porter, Jr.[1]

Introduction

Because nut grass is a weed problem, there is need for knowledge of the factors affecting the sprouting of tubers following conditions in which they are dormant. Similarly an understanding of the mechanisms involved in the control of dormancy along with information on the metabolism of tubers at different stages of growth is needed. The purpose of this series of studies was to obtain information on some of those problems. The purpose of the present study was to determine the response of nut grass to several oxygen levels as measured by germination habits and respiration of tubers.

Literature Review

Pfeffer (7) stated that most aerobic plants fail to grow in an atmosphere in which the oxygen concentration is below 3 per cent, and that a level of oxygen above that found in air often retards growth. Valmis and Davis (13) found that the growth of rice roots attained a maximum in 3 per cent oxygen, and that this growth was greater than in air. The growth of barley shoots was greater in air than in gas mixtures containing less oxygen. Taylor (10) and Lin (6) have shown that growth of young shoots and roots of wheat and rice is progressively reduced in oxygen levels below 21 per cent, but to a less extent for the shoots than the roots. Leonard and Pinckard (5) reported that cotton root growth was greater in air than in mixtures containing lower oxygen levels. The optimum carbon dioxide level was between 0 and 15 per cent when oxygen was 21 per cent. The absence of carbon dioxide failed to influence root growth, but concentrations above 45 per cent prevented all root growth. Oxygen at 100 per cent reduced root elongation.

According to James (4), Watson presented data on the relation of oxygen consumption to concentration. He found that oxygen uptake of Bramley seedling apples was greater at 25 and 30 per cent oxygen levels than at 20 per cent or below. Rice seedlings, barley seedlings, and onion roots showed the same general response.

Stich (9) was one of the earliest to examine the effects of reducing oxygen concentration upon the respiratory quotient (R. Q.). He found at 9 per cent oxygen the R. Q. changed very little from the air value for wheat, maize, and pea seedlings, but a considerable rise occurred at 3 per cent oxygen. The R. Q.s for narcissus bulbs were close to 1.0 in air or 10.2 per cent oxygen, but were 2.4 in 7.5 per

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cent oxygen. Similar results have been reported for Granny Smith apples (3), tomatoes (2), and carrot slices (11).

Allsopp (1) observed no anatomical differences in the structure of growing shoot apices of *Drypoteris aristata* and of plantings developing from detached meristems of rhizomes of *Onoclea sensibilis* and *Matteuccia struthiopteris* when kept in gas mixtures containing 6, 11, 21, and 45 per cent oxygen in nitrogen. In undiluted oxygen, sections of rhizomes of *Matteuccia* and *Onoclea* developed periderm-covered, cushion-like growths from the detached meristems.

MATERIALS AND METHODS

Nut grass grown in pots in the greenhouse was the source of the tubers. For each experiment several pots were emptied and the soil was removed with water. Single dormant tubers were separated from the tuber system, and three and five dormant tubers connected by rhizomes also were separated from the system. These materials were dipped into a 1 per cent aqueous solution of Vancide 51 and blotted with paper towels.

I. Germination of single tubers and three and five tubers connected by rhizomes in gas mixtures.

The gas mixtures used were 1, 10, and 20 per cent oxygen in combination with 1, 5, 10, and 15 per cent carbon dioxide, with nitrogen as the inert gas. Oxygen, carbon dioxide, and nitrogen at 100 per cent also were used. The gas mixtures were prepared in tanks, and a separate tank was used for each mixture. For each treatment four 125 ml Erlenmeyer flasks connected by tubing were used. Two flasks contained three single tubers each, and the other two flasks contained one chain of three tubers. Each treatment was replicated three times, and the complete test was triplicated. In a different experiment each of the four flasks contained one chain of five tubers. Each treatment was duplicated. The tubers were maintained on moist filter paper during the experimental period. The system of four flasks was evacuated for three minutes with an ordinary laboratory water pump. A particular gas or gas mixture was then flushed into the system to refill it once a day for 17 days. The system was sealed with a clamp on Tygon tubing and kept in the laboratory.

At the end of the 17th day, counts were made on the germination of single tubers as well as tubers in the chains. The data reported for single tubers are averages for the experiments. The flasks which contained the single tubers were then opened and left in air for 11 days. During this period the tubers were kept on moist filter paper. The purpose of the above treatment was to determine the viability of tubers which had failed to germinate in low oxygen levels.

After the patterns of germination had been determined for chains containing five tubers the dormant tubers were separated from the chains and returned to the flasks. These tubers remained in air for four days, and the germination of individual tubers was determined.

II. Respiration of dormant and germinated tubers in oxygen gradients.

Oxygen uptake and carbon dioxide evolution were determined by using a Warburg respirometer and conventional methods described by Umbreit et al. (12). The temperature was 25° C.

Gas exchange was determined in 1 and 10 per cent oxygen in mixture with nitrogen and in air, and in 100 per cent oxygen. Undiluted oxygen or a gas mixture was flushed through the Warburg flasks. Approximately one liter of gas or gas mixture was flushed through each flask after equilibration. The stopcocks of the manometers and flasks were closed, and the gas exchange was measured. Four Warburg flasks were employed for oxygen uptake and carbon dioxide evolution. Each flask contained four tubers suspended in 3 ml of buffer solution at pH 6.0 which was 0.05 M and 0.02 M with regard to potassium phosphate and sucrose, respectively. Immediately following each determination, the tubers for a particular treatment were removed from the Warburg flasks and placed on moist filter paper in four 125 ml Erlenmeyer flasks. The flasks were connected with Tygon tubing and once a day for six days the system was evacuated with an ordinary water pump for three minutes. A particular gas or gas mixture, that is, the one in which respiration was determined, was then flushed into the system to refill it, and the system was sealed with clamps. On the 6th day, gas exchange was determined in the same manner as described for dormant tubers.

RESULTS AND DISCUSSION

I. Germination of single tubers and three and five tubers connected by rhizomes in gas mixtures.

Single tubers. Oxygen levels below 20 per cent reduced germination. Approximately 80 per cent germination occurred in 20 per cent oxygen and increasing levels of carbon dioxide appeared to slightly enhance germination (Figure 1). Tubers germinated about 8 per cent less in 10 per cent oxygen, and increasing the carbon dioxide concentration appeared to slightly reduce germination. Increasing carbon dioxide concentration in combination with 1 per cent oxygen caused a sharp decline in per cent germination for these treatments.

Tubers exposed to 100 per cent carbon dioxide or nitrogen failed to germinate.

Oxygen at 100 per cent induced 95 per cent germination. This was 15 per cent greater than those tubers exposed to 20 per cent oxygen. At the end of the 6th day, 78 per cent of the tubers which were in 100 per cent oxygen had germinated, whereas only 44 per cent had germinated in air. This possibly is indicative of an oxidase enzyme responding markedly to oxygen concentration. Tubers exposed to air for 11 days, after being under treatment for 17 days, showed an average germination of 90 per cent, for all treatments. This showed that tubers remained viable under 1 and 10 per cent

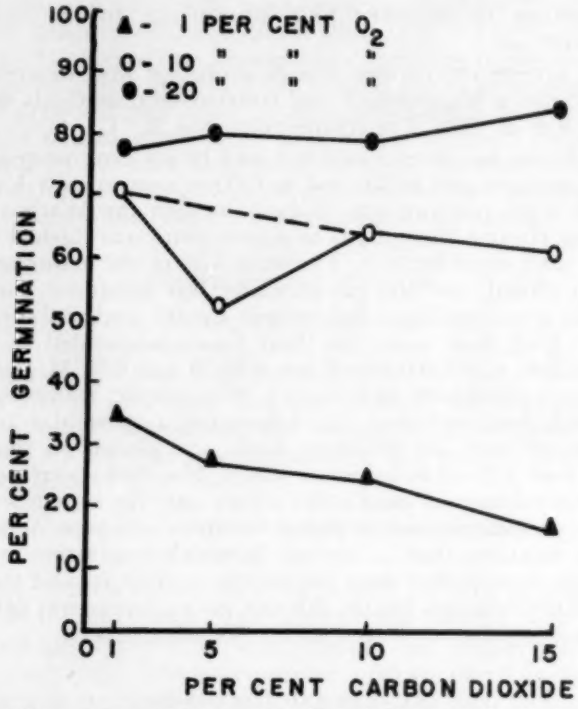

Figure 1. The influence of 1, 10, and 20 per cent oxygen in mixture with 1, 5, 10, and 15 per cent carbon dioxide upon the germination of single tubers.

oxygen, even though the per cent germination was low under these treatments.

Three tubers in a chain. To determine if different oxygen levels influenced dominance in the chain, the number and position of tubers in the chain which germinated were recorded. The chains had been broken at random from the tuber system, so that stage of development of the tubers was not known in any of the chains. It was found that all possible patterns of germination occurred under the different oxygen levels (Table 1). Under the lowest oxygen level, the largest number of chains showed all tubers dormant (Tables 1, 2). There was a significant shift in 10 per cent oxygen to a larger number of patterns which showed two and three tubers germinated. Significantly more tubers germinated in 20 per cent oxygen than in 10 per cent oxygen (Tables 1, 2). Carbon dioxide level did not significantly influence germination in either oxygen concentration.

Five tubers in a chain. At the lowest oxygen concentration the largest number of chains showed complete dormancy (Tables 3, 4). This pattern was followed by one in which a single tuber germinated. However, in 10 per cent oxygen there was a slight shift to patterns

Table 1. The influence of various oxygen and carbon dioxide concentrations upon the number and position of germination of three tubers in a chain.

Chain patterns	Number of chains which showed each pattern of germination in treatment number[a]											
	1	2	3	4	5	6	7	8	9	10	11	12
O-O-O[b]	5	6	12	9	1	2	1	5	0	1	1	1
X-O-O	7	5	2	5	5	4	6	1	2	4	1	2
O-X-O	3	0	2	0	1	0	0	2	4	1	4	2
X-O-X	1	1	0	0	1	3	3	3	2	3	4	2
X-X-O	1	3	1	4	5	4	4	3	5	4	6	3
X-X-X	1	3	1	0	5	5	4	4	5	5	2	8

[a]Treat. No. | Gas mixtures Per cent O_2 | CO_2 | N_2

Treat. No.	O_2	CO_2	N_2
1	1	1	98
2	1	5	94
3	1	10	89
4	1	15	84
5	10	1	89
6	10	5	85
7	10	10	80
8	10	15	75
9	20	1	79
10	20	5	75
11	20	10	70
12	20	15	65

[b]The O represents the dormant tubers and the X represents the germinated tubers.

Table 2. The influence of oxygen level upon the number of germination of three tubers in a chain.

Number germinated out of three	Number of chains of three tubers with the indicated number of tubers germinated following exposure to		
	1% O_2	10% O_2	20% O_2
0	42	9	3
1	24	19	20
2	11	26	39
3	5	18	20

which showed mostly two and three, and in some cases four tubers germinated. Carbon dioxide concentration appeared to have no predominating effect upon the patterns. The shift from a pattern which showed one tuber germinated to patterns which showed two, three, and four, was striking in 20 per cent oxygen (Tables 3, 4, and 5).

The dormant tubers in the chain described above germinated when they were separated and exposed to air for four days (Table 5). This demonstrates that the dominant tuber or tubers present in a chain apparently control the germination of the dormant tubers.

Dominance was less under high oxygen levels and greater under low oxygen levels. This might be due to changes in auxin available to the germinated tuber under 10 and 20 per cent oxygen. It is possible that the oxidative destruction of auxin is higher under 10 and 20 per cent oxygen. This possible destruction of auxin would reduce the quantity available to exert dominance. The reverse situation could have occurred under 1 per cent oxygen. This concept is supported by Ranson and Parija (8) who have shown that when coleop-

Table 3. The influence of various oxygen and carbon dioxide concentrations upon the number and position of germination of five tubers in a chain.

Chain patterns	Number of chains which showed each pattern of germination in treatment number[a]											
	1	2	3	4	5	6	7	8	9	10	11	12
O–O–O–O–O[b]	3	1	1	2			2					
X–O–O–O–O	2	1	2	2	2		1	1	1			
O–X–O–O–O	2	1		1	2	1	1	1				
O–O–X–O–O			1						1			
X–X–O–O–O								2			1	
O–O–X–X–O		1	1	1	1	2		1	1	1		1
O–X–O–X–O	1						1		1			1
O–X–O–O–X		2	2	1				1				2
O–O–X–O–X					2		2					
X–O–O–O–X								1			2	
O–O–X–X–X						1						1
O–X–X–X–O				1	1	1					1	
O–X–O–X–X		1	1					1				
O–X–X–O–X									2	2		1
X–O–O–X–X									2	1		
X–O–X–O–X		1								1	2	
O–X–X–X–X										1	1	1
X–O–X–X–X						2				1	1	1
X–X–O–X–X						1	1			2		
X–X–X–X–X												

[a]Treat. No.	Gas mixtures Per cent O_2	CO_2	N_2
1	1	1	98
2	1	5	94
3	1	10	89
4	1	15	84
5	10	1	89
6	10	5	85
7	10	10	80
8	10	15	75
9	20	1	79
10	20	5	75
11	20	10	70
12	20	15	65

[b]The O represents the dormant tubers and the X represents the germinated tubers.

Table 4. The influence of oxygen level upon the number of germination of five tubers in a chain.

Number germinated out of five	Number of chains of five tubers with the indicated number of tubers germinated following exposure to		
	1% O_2	10% O_2	20% O_2
0	7	2	0
1	12	9	2
2	9	12	10
3	4	4	13
4	0	3	7
5	0	0	0

tiles of certain cereals were transferred from air to 3.5 and 8.5 per cent oxygen the rate of growth was often doubled. Root growth decreased as the oxygen concentration was reduced below 21 per cent.

II. Respiration of dormant and germinated tubers in oxygen gradients.

This study was conducted to obtain respiratory data on single dor-

Table 5. The germination of dormant tubers separated from a chain of five tubers.

Gas mixtures per cent			Number of tubers dormant in 8 chains	Number of tubers germinated—under air for four days
O_2	CO_2	N_2		
1	1	98	34	34
1	5	94	27	27
1	10	89	28	27
1	15	84	30	30
			119	118
10	1	89	29	28
10	5	85	24	23
10	10	80	27	26
10	15	75	25	25
			105	102
20	1	79	15	12
20	5	75	10	9
20	10	70	13	13
20	15	65	20	18
			58	52
LSD 5%			13.7	
LSD 1%			19.4	

mant tubers, and germinated tubers which were kept in varying oxygen levels. Germinated tubers showed an increase in oxygen uptake when the oxygen level was increased. Carbon dioxide evolution was higher than oxygen uptake in tubers exposed to 1 and 10 per cent oxygen, but the gas exchange was near unity in 20 and 100 per cent oxygen (Figure 2B, C). The total gas exchange was higher in 20 and 100 per cent oxygen.

Oxygen uptake for dormant tubers was low in 1 per cent oxygen, but it increased in 10, 20 and 100 per cent oxygen (Figure 3A). Carbon dioxide evolution was high at the 1 per cent oxygen level and decreased as the oxygen level was increased. The R. Q.s for dormant tubers in 1, 10, 20, and 100 per cent oxygen were 4.5, 3.5, 3.8 and 1.5, respectively (Figure 2C). The total gas exchange increased as the oxygen level increased (Figure 2A).

The fact that more dormant single tubers germinated in 100 per cent than in 20 per cent oxygen would suggest that oxygen was one factor that limited germination. Respiration and germination were less in 1 and 10 per cent oxygen than in 20 per cent oxygen. Thus there appears to be a correlation between germination and aerobic respiration. On the other hand, the inhibitory action of possible anaerobic carbon dioxide plus high external carbon dioxide levels would possibly account for the low percentage of germination of tubers under such conditions.

SUMMARY

Studies to determine the germination and respiration of nut grass as influenced by oxygen levels are reported. These studies showed:

1. After 17 days of exposure the per cent germination of tubers

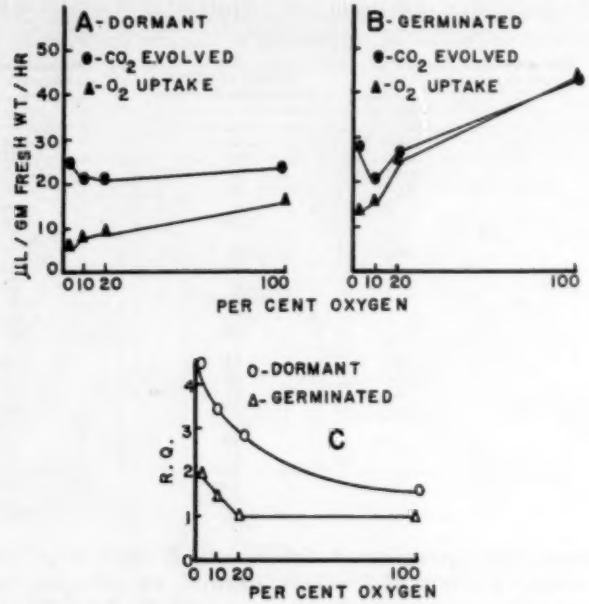

Figure 2. A and B—The influence of oxygen concentration upon the respiration of dormant and germinated tubers, respectively. C—The R.Q.s for the dormant and germinated tubers.

was 34, 70, and 77 respectively, for 1, 10, and 20 per cent oxygen in mixture with 1 per cent carbon dioxide. As the carbon dioxide concentration increased, germination decreased in 1 and 10 per cent oxygen and slightly increased in 20 per cent oxygen. At the 15 per cent carbon dioxide level in mixture with 1, 10, and 20 per cent oxygen, the per cent germination was 17, 60, and 83, respectively. Ninety-five per cent of single tubers germinated in 100 per cent oxygen.

2. When chains of tubers were exposed to the gas mixtures mentioned above, more tubers germinated in the higher oxygen levels than in the lower levels. In many instances, all of the tubers germinated at the high oxygen levels, but at the low oxygen level a greater number of the tubers remained dormant. This showed that high oxygen levels depressed chain dominance. When dormant tubers were separated from a chain in which certain tubers were germinated and exposed to air, they germinated. This demonstrates that the germinated tubers exerted dominance in the chain.

3. Respiration of dormant tubers increased as the oxygen level was increased from 1 to 100 per cent. The same was true for the germinated tubers; however, the total gas exchange was greater from the germinated tubers. The R.Q.'s for the dormant tubers were 2.9 and 1.5, respectively, at the 20 and 100 per cent oxygen levels, but

they were unity for the germinated tubers at these oxygen levels. The R.Q.'s for dormant and germinated tubers were indicative of anaerobic respiration in 1 per cent oxygen and to a less degree at the 10 per cent level.

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The Metabolism of Nut Grass (*Cyperus rotundus* L.) II. The Respiratory Quotient and Its Relation to Storage Materials and Some Terminal Enzymes

R. D. Palmer and W. K. Porter, Jr.[1]

To obtain information on the metabolism of nut grass tubers at different stages of growth, this study purposed to determine certain metabolic pathways of tubers at different stages of germination, which might aid in determination of the most sensitive stage to herbicides.

Literature Review

The respiratory quotient (R.Q.) has been used to determine the type of metabolism operating in a plant. Although carbohydrates undoubtedly are the most frequent and the major substrate for the reactions of respiration, other substrates also may be utilized, particularly the organic acids and fatty acids. In complete respiratory oxidation of hexose, the R.Q. is unity. In complete oxidation of organic acids, the R.Q. is greater than one, usually 1.3 to 1.5. Conversely, in the complete oxidation of fatty acids the R.Q. is less than one (2).

The terminal enzyme system is coupled with the substrate oxidation whether the substrate is carbohydrates, organic acids, or fats. Eddy et al. (3) reported that some plants may use one terminal oxidase system and later change to another system. Albaum and Eichel (1) reported that germinating oats initially used the cytochrome system but later shifted to ascorbic acid oxidase system. Goddard (4) reported similar results for wheat. Albaum and Eichel (1) found a change occurred in the metabolic substrate from fats to sugars with the increased ascorbic acid oxidase activity in germinating lettuce seed, but other terminal oxidases became more active than ascorbic acid oxidase as germination proceeded.

Although polyphenoloxidase and ascorbic acid oxidase have been found in a number of plants, neither apparently is the universal oxidase. Eddy et al. (3) reported that system other than ascorbic acid oxidase may oxidize ascorbic acid. Robinson and Nelson (13) showed that the L–tyrosine–tyrosinase system used ascorbic acid as a reductant, and Hattori and Siroga (5) found that ascorbate was oxidized by 3,4–dihydroxyphenylalanine (dopa).

Materials and Methods

Nut grass grown in pots in the greenhouse served as a source of experimental material (10).

I. General procedure for measurement of oxygen uptake and carbon dioxide evolution.

Gas exchange determinations were carried out by conventional Warburg methods (15) at 25° C.

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Respiration of whole tubers during germination. Four Warburg flasks each containing four tubers were employed for measuring the oxygen uptake and carbon dioxide evolution. Each flask received 3 ml of a pH 6.0 buffer composed of potassium phosphate and sucrose at 0.05 M and 0.02 M, respectively. A gas exchange measurement was made on dormant tubers and on germinating tubers each day for 6 days and again on the 10th day. Between determinations, the tubers were kept on moist filter paper in Petri plates. To determine growth from the time of dormancy, weights of the tubers were taken prior to each determination. The data presented are average values of two experiments.

Anaerobic respiration of whole tubers. Carbon dioxide evolution was measured in 100 per cent nitrogen. The flasks were flushed as previously described (10). Duplicate flasks were employed, and the test was duplicated. Each flask contained four dormant tubers and 3 ml of the buffer mentioned above.

Respiration of slices 2–3 mm thick. Some 35–40 slices from dormant tubers were placed in the Warburg flasks which contained 3 ml of the buffer mentioned above. Duplicate flasks were employed and the test was duplicated.

II. Krebs cycle studies.

Preparation of "particles." Forty to 50 gm of tubers were sliced 3–4 mm thick. The slices were placed in a pre-chilled chamber of the Omni mixer which contained 250 ml of cold 0.5 M sucrose and 0.1 M potassium phosphate buffer at pH 6.0. The chamber was submerged in a constant temperature bath at 2° C. The homogenizer was run for five minutes at 80 volts. Following homogenization, the suspension was separated by differential centrifugation. The temperature was maintained below 10° C. during the following procedure: The suspension was filtered through one layer of cheese cloth, and the filtrate was centrifuged for 10 minutes at 1000 x g. The supernatant was centrifuged at 20,000 x g for 15 minutes. The precipitate was resuspended in the same buffer and centrifuged at 20,000 x g for 30 minutes. The precipitate was then suspended in 10 ml of 0.4 M sucrose and 0.02 M potassium phosphate buffer at pH 6.0 and frozen. This suspension was designated as the "particle" suspension. When employed, the suspension was quickly thawed and used with or without dilution.

Dehydrogenase determination. Dehydrogenase determinations were made by using the above "particle" suspension. The activity was determined in Thunberg tubes by determination of the rate of anaerobic reduction of sodium 2,6–dichlorophenolindophenol (DIP) as described by Umbreit et al. (16), and Price and Thimann (11). Colorimetric measurements were made by using a 630 filter. Specific activity (S.A.) was computed by obtaining the endogenous reduction of DIP in the presence of buffer and a 0.5 ml aliquot of "particle" suspension. Total reduction was determined in the presence of "particles," buffer and co-factors. Endogenous reduction was subtracted from total reduction and divided by the total nitrogen in the 0.5 ml of "particle" suspension. This value was multiplied by 100.

The final concentrations of components for isocitric and succinic dehydrogenase activities are listed below: Succinate, 2×10^{-2} M; DIP, 5×10^{-5} M; $MgSO_4$, 2×10^{-3} M; potassium phosphate and sucrose, 2×10^{-2} M and 0.4 M, respectively, and a final pH of 6.8. Glass distilled water was added to bring the volume up to 4 ml. For isocitric dehydrogenase activity, the same components were used as mentioned above except isocitrate at the same concentration was used instead of succinate, and triphosphopyridine nucleotide (TPN) at 1×10^{-3} M was used.

Oxidase determinations. The various co-factors and substrates were placed in the main compartment of the Warburg flask. A 0.5 ml aliquot of the "particle" suspension was placed in the side arm and tipped into the main compartment after equilibration. The final concentrations of the components for the oxidases are listed below: Isocitrate 1×10^{-2} M; succinate, 1×10^{-2} M; a–ketoglutarate, 2×10^{-2} M; a–ketoglutate, 2×10^{-2} M, plus malonate at 1×10^{-2} M. For each oxidase, the complete system contained in addition to one of the above substrates the following components: Adenosine diphosphate, 2×10^{-3} M; adenosine triphosphate, 1×10^{-3} M; Diphosphopyridine nucleotide, 5×10^{-4} M; TPN, 5×10^{-4} M; cocarboxylase, 1.23×10^{-4} M; cytochrome c, 6.83×10^{-5} M; $MgSO_4$, 2×10^{-2} M;

and potassium phosphate and sucrose, 2 x 10^{-2} M and 0.4 M, respectively, and a final pH of 6.7. Glass distilled water was added to bring the volume up to 1.7 ml.

III. Estimation of enzyme activities and inhibition.

Preparation of homogenates. Dormant tubers were allowed to germinate in a moist chamber. For the enzyme source, dormant tubers and actively growing tubers were selected every two days over a 8-day period. For each determination, five gm of tubers were sliced 3–4 mm thick. These slices were placed in a Waring blender containing 50 ml of 0.05 M potassium phosphate buffer of pH 6.0 and were homogenized for two minutes at a temperature below 10° C. After blending the crude homogenate was filtered through two layers of cheese cloth, and the filtrate was centrifuged at 1000 x g for 10 minutes at 2° C.

Ascorbic acid oxidase activity. A 2 ml aliquot of homogenate was placed in the main compartment of the flask. The ascorbate was added to the side arm and tipped in following equilibration. The final concentration of ascorbate in the reaction mixture is reported.

Catecholase and its inhibition with ascorbic acid. A 2 ml aliquot of homogenate was placed in the main compartment and a 0.5 ml aliquot of catechol in buffer was placed in the side arm and tipped in after equilibration.

In order to demonstrate the inhibition of catecholase with ascorbic acid, a 0.5 ml aliquot of ascorbic acid and catechol, respectively, were placed in separate side arms of the flask. These materials were tipped into a homogenate prepared from dormant tubers. The ascorbate was used at variable rates while the catechol rate was constant, and only the final concentrations are reported. The ascorbate was prepared in buffer and adjusted to pH 6.0 with NaOH. Duplicate flasks were employed for each treatment, and the test was duplicated.

Dopa (3,4–dihydroxyphenylalanine) oxidase and its inhibition by ascorbic acid. A 2 ml aliquot of homogenate from dormant tubers was placed in the Warburg flask. A 0.5 ml aliquot each of L–dopa and ascorbate at pH 6.0 in buffer were placed in the side arms. Ascorbic acid was used at variable rates, and L–dopa was used at one rate, and only the final concentrations are reported. These materials were tipped simultaneously into the homogenate and the oxygen uptake was recorded. One-half hour after tip in, samples of a companion reaction mixture were utilized for determining adsorbance at 470 mu. At the end of one hour, a sample from all flasks was used for adsorbance determinations. Specific activity (S. A.) was determined by substracting the adsorbance change during the reaction period of the enzyme without added substrate from the adsorbance change in the presence of enzyme and L–dopa, on L–dopa and ascorbate, and dividing by the quantity of nitrogen in the 2 ml of homogenate. The value obtained was multiplied by 100 (Bailey[2]).

IV. Preparation of dry tissue.

Fresh whole tubers employed in experiments were dried in a convection oven set at 80° C. for 24 hours. Dry weights were determined, and the tubers were ground in the Wiley mill equipped with a 60 mesh screen. The material was kept in dry stoppered flasks and utilized in certain analyses.

Tissue slices were taken from the Warburg flasks and washed to remove the buffer. The tissue was dried as described above and dry weight was determined.

V. Chemical methods.

Total nitrogen. Nitrogen in homogenates, the "particle" suspension and dry ground material from whole tubers, was determined by digesting with a Na_2SO_4, H_2SO_4 and $CuSeO_3$ mixture. The NH_3 was trapped in H_3BO_3 and titrated with H_2SO_4 (6). A modified Pregl-Parnas-Wagner micro apparatus was employed for the liberation of the NH_3 from the digested sample (7).

Fats and soluble carbohydrates. Extractable fats were estimated according to the procedure of Jacobs (7). Soluble carbohydrates were extracted by the method

[2]Bailey, M. C. The biochemistry of melanin formation in shrimp (A dissertation). Louisiana State University. 1958.

of Jacobs (7) and estimated by the anthrone method described by Umbreit et al. (16).

Organic acids. Estimation of total organic acids, and malic and citric acids, was carried out according to the procedure of Pucher et al. (12). Oxalic acid was estimated by the method of Palmer (9).

Ascorbic acid and dehydroascorbic acid. Ascorbic acid was estimated by the procedure of Morell (8). Dehydroascorbic acid was estimated according to the procedure of Roe et al. (14).

RESULTS AND DISCUSSION

1. Respiration and growth during germination.

To obtain more information about the respiration of nut grass, gas exchange was followed during germination. Carbon dioxide evolution initially was considerably greater than oxygen consumption (Figure 1A). Dormant tubers had an R. Q. of 2.1 (Figure 1B). However, the rate of oxygen consumption increased with time and an R. Q. of unity was obtained by five and one-half days (Figure 1A,

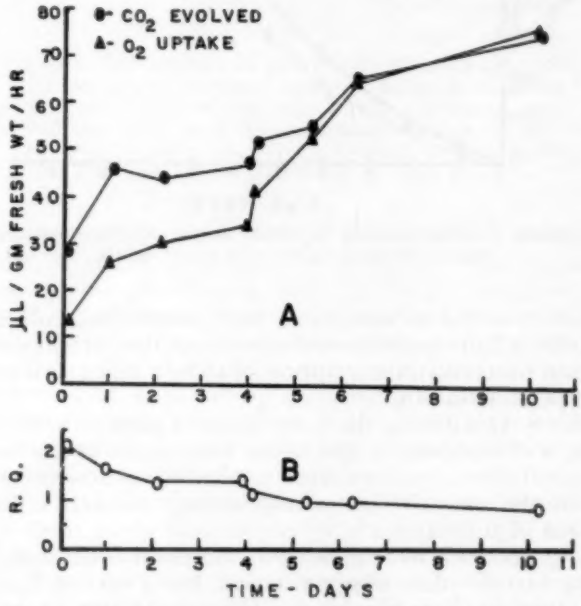

Figure 1. A—Changes in oxygen consumption and carbon dioxide evolution by tubers during germination in water. B—The R. Q.'s derived from the gas exchanged during this period.

B). At this point, the tubers were in the stage of maximum growth (Figure 2). The total gas exchange also increased with time over the experimental period (Figure 1A). Growth in fresh weight also increased over the experimental period (Figure 2). The high R. Q.'s obtained in early stages of germination suggested that an organic

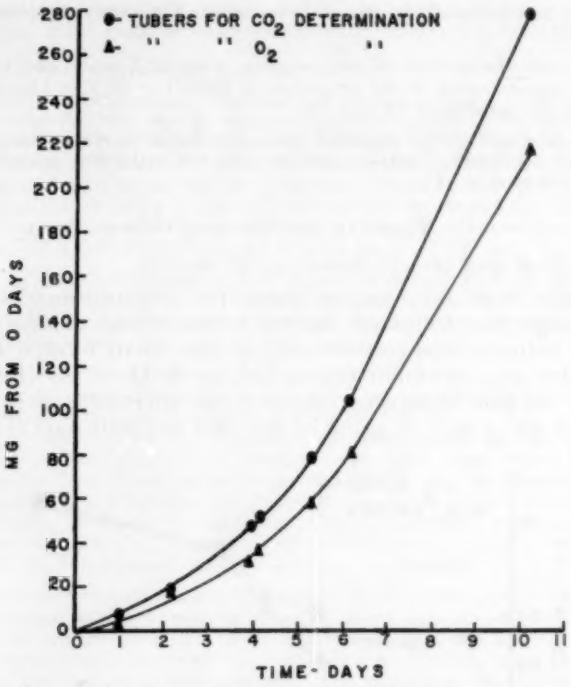

Figure 2. The increase in fresh weight of germinating tubers involved in the respiratory study.

acid metabolism was in operation, and possibly anaerobic respiration contributed to the evolution of some of the carbon dioxide. As germination proceeded, there appeared to be a rapid shift to a carbohydrate type metabolism.

Since the R. Q.'s during the early stages of growth were indicative of organic acid metabolism, and in the later stages indicated a carbohydrate metabolism, analyses were conducted to determine possible changes in the quantity of certain storage materials during the germination of tubers.

Total organic acids were high in the dormant tuber and increased during the first two days of germination, but a very striking decline occurred from two to four days. This was followed by a rapid increase from 6 to 10 days (Figure 3A). Citric acid, malic acid, and oxalic acid were constant over the experimental period, but citric acid was higher in quantity than malic acid and the latter was higher than oxalic acid (Figure 3A).

Fats gradually declined in quantity until the 6th day, and increased to the 8th day (Figure 3B). Soluble carbohydrates showed a gradual decline after two days (Figure 3B). Total nitrogen showed a decline from the dormant tuber to four days, and increased slightly to a higher level (Figure 3C).

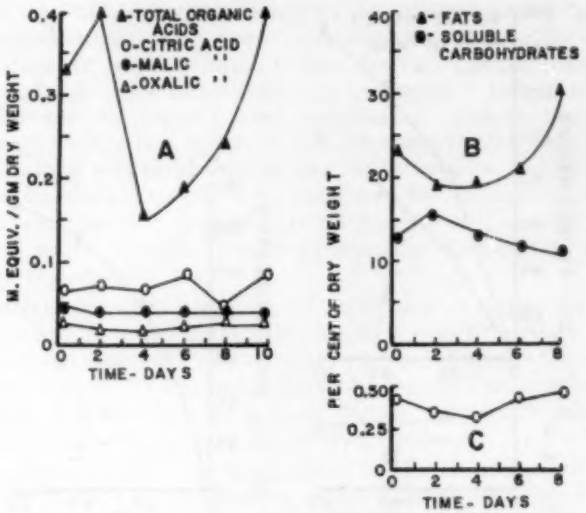

Figure 3. The influence of germination of tubers upon the changes in certain storage materials during germination. A—The quantity of total organic acids, citric acid, malic acid, and oxalic acid; B—The quantity of fats and soluble carbohydrates; and C—The quantity of total nitrogen. The tubers were germinated in water.

The changes in organic acids and carbohydrates coincide with the changes in the R. Q.'s. This is suggestive that organic acid metabolism was the case during the early stages of germination, and it appears that carbohydrates were metabolized in the later stages of growth. The primary organic acids utilized in greater quantity would be those other than malic, citric, and oxalic. Nitrogen and fats appeared to be used during germination. Total organic acids, fats, and nitrogen were synthesized in the latter stages of germination. These materials probably were synthesized with new growth or from storage carbohydrates.

To test the idea that oxygen gradients within the dormant tuber might have been partly responsible for the high R. Q.'s, tubers were sliced 2–3 mm thick, and the respiration of these slices was determined. Total gas exchange was much greater by the slices than by the dormant tubers (Figures 4A and IA). However, the R.Q. was 1.5 for the tissue slices (Figure 4B). The R. Q. for the slices would indicate perhaps an organic acid metabolism. Since the whole dormant tuber does have a high anaerobic respiration (Figure 4C), it is believed that some anaerobic respiration does take place in the dormant tuber. The anaerobic carbon dioxide possibly would account for that evolved above the quantity to give an R. Q. of 1.5.

To study the enzymes responsible for the decarboxylation of organic acids in the Krebs cycle, "particles" from dormant and germinated tubers were employed. Isocitric and succinic dehydrogenase

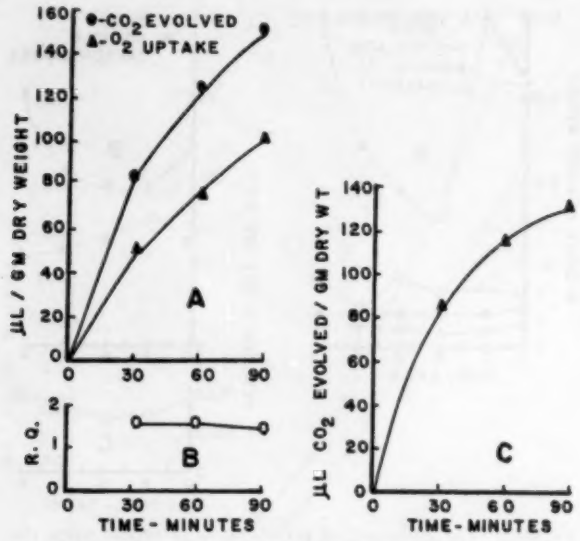

Figure 4. A—Oxygen uptake and carbon dioxide evolution from tissue slices 2–3 mm thick. B—The R. Q.'s from the data in A. C—Anaerobic carbon dioxide measured from whole dormant tubers.

activities were estimated in the dormant and germinated tubers. The results of the activities are presented in Figure 5. It may be seen that isocitric dehydrogenase was very active in dormant tubers, but the activity was less in germinated tubers. A point of significance is that isocitric dehydrogenase activity decreased with germination, whereas succinic dehydrogenase increased in activity (Figure 5). The

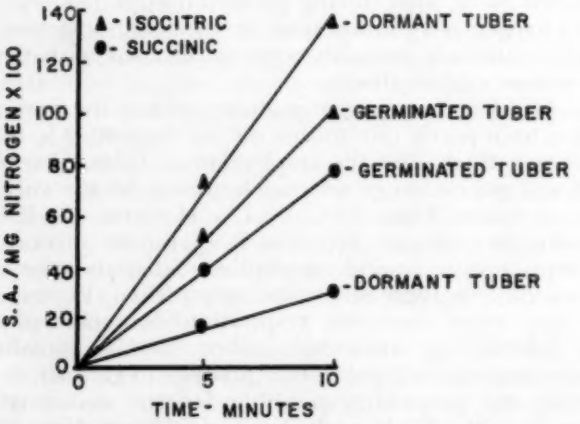

Figure 5. Isocitric and succinic dehydrogenase activities on "particles" from dormant and germinated tubers.

total activities of isocitric and succinic dehydrogenase on "particles" from dormant tubers were approximately the same as the activities on "particles" from germinated tubers. It is concluded that the predominance of isocitric dehydrogenase in dormant tubers is perhaps responsible for the organic acid metabolism. The carbon dioxide evolution probably is a result of the decarboxylation of the oxalosuccinate which was produced by the active isocitric dehydrogenase. Furthermore, the a–ketoglutarate produced by the previous reaction probably is oxidatively decarboxylated. Since the "particles" demonstrated an active succinic dehydrogenase, the Krebs cycle probably was in operation.

It is interesting that an R. Q. of unity was obtained in the germinated tubers at 6 days, and that isocitric dehydrogenase and succinic dehydrogenase were about equally active (Figures 1B, 5). Thus, there appears to be a correlation between the shift to a carbohydrate metabolism and the shift to approximately equal activities of isocitric dehydrogenase and succinic dehydrogenase.

To further examine the Kreb cycle in the dormant tubers, succinic, isocitric, and a–ketoglutaric oxidase activities were measured with "particles." Succinate was oxidized two-fold greater than isocitrate, and a–ketoglutarate was oxidized slightly less than succinate. Malonate inhibited the oxidation of a–ketoglutarate by 62 per cent (Table 1). It is concluded that the oxidation of isocitrate and

Table 1. The oxidation of Krebs cycle intermediates by "particles" isolated from dormant tubers.

Substrate	Final concentration (M)	Microliters of O_2 uptake mg N/50 min
Isocitrate	0.01	154
Succinate	0.01	338
a-Ketoglutarate	0.02	292
a-Ketoglutarate plus malonate	0.02 0.01	112

a–ketoglutarate proceeded through the common intermediate succinate because malonate is an effective inhibitor of succinate. This demonstrates that this area of the Krebs cycle was in operation, and probably is in some way responsible for the organic acid metabolism of the dormant nut grass tuber.

II. The ascorbic acid inhibition of catecholase and dopa oxidase during germination.

Because the R. Q.'s and dehydrogenases changed during germination, it was considered worthwhile to study some terminal oxidases. The homogenates were prepared from the dormant and germinating tubers. When the oxidation of catechol and ascorbate were examined in the dormant tuber, catechol oxidation was 5.9 times higher than ascorbate oxidation (Figure 6). As germination proceeded, ascorbate

oxidation increased with time; and at about five and one-half days, the oxidation of catechol and ascorbate oxidation were equal. The sum for the oxidation of the two substrates was approximately the same over the experimental period until five and one-half days (Figure 6). On the 8th day, however, ascorbate oxidation was 3.9 times

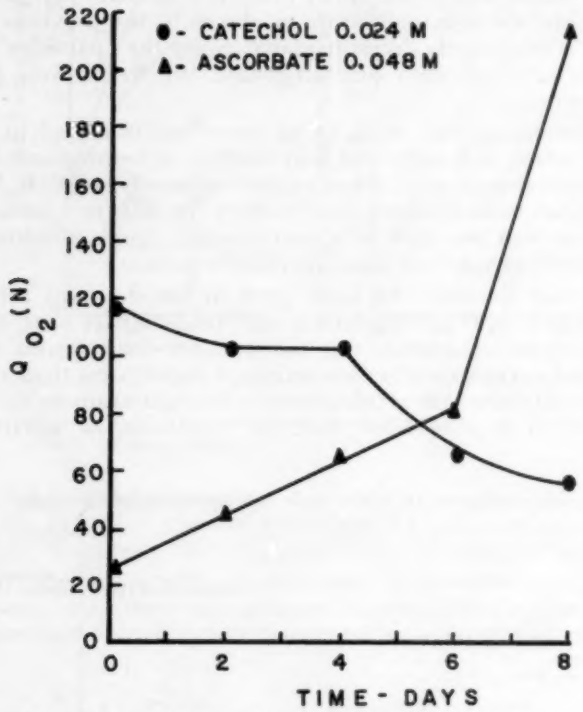

Figure 6. The oxidation of catechol and ascorbate in homogenates prepared from tubers during germination in water.

greater than catechol oxidation. It was thought that the changes in catechol and ascorbate oxidation were partly the oxidation of some natural reducing substance in the tissue, perhaps reduced ascorbate.

Ascorbate analyses were conducted on tubers during germination. Ascorbate was at the highest level in dormant tubers, whereas dehydroascorbate was low (Figure 7A). The ratio of ascorbate to dehydroascorbate was 3.5 in the dormant tuber (Figure 7B). However, as germination proceeded, ascorbate decreased and dehydroascorbate increased; and by the 8th day of germination, the ratio of ascorbate to dehydroascorbate was 1.5 (Figure 7A, B).

It was believed that the o–quinone formed from the oxidation of catechol was reduced in the presence of ascorbate; consequently,

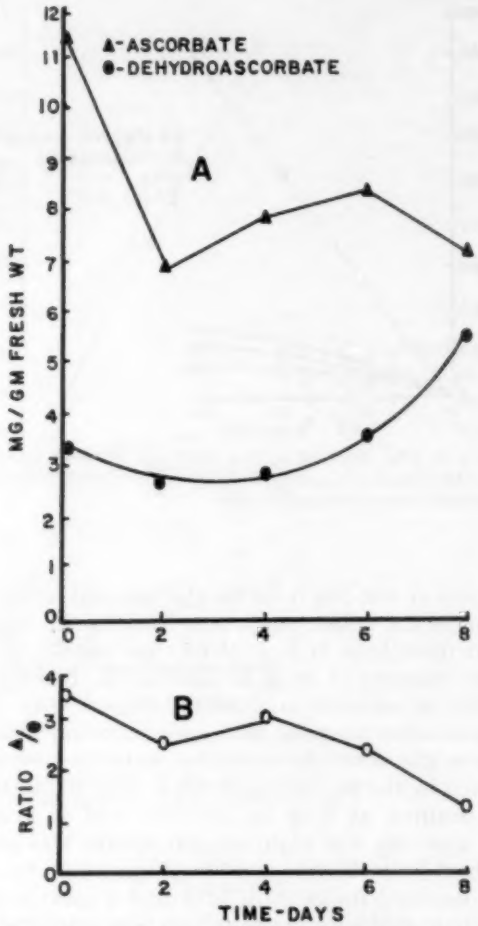

Figure 7. A—The quantity of ascorbate and dehydroascorbate determined from tubers during germination in water. B—The ratio of ascorbate to dehydroascorbate during germination.

catecholase would absorb oxygen in this system and ascorbate would be oxidized. The quantity of oxygen uptake would be higher in a homogenate from the dormant tuber which contained more ascorbic acid than the germinated tuber. This was confirmed by determining the rate of oxygen uptake in a homogenate from a dormant tuber. Typical results are shown in Figure 8. Oxygen uptake was roughly proportional to the ascorbate level. It was noticed that no color developed as the result of catechol oxidation as long as there was ascorbic acid in the reaction mixture.

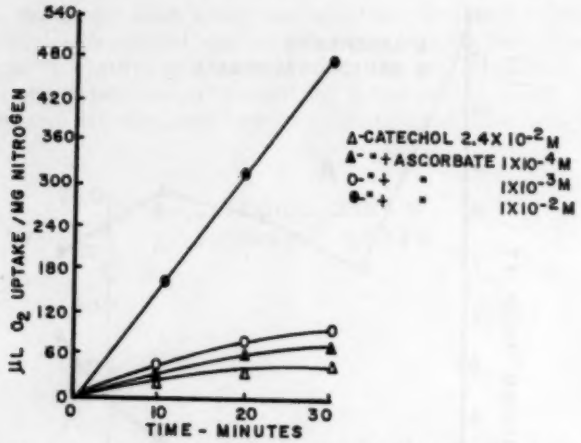

Figure 8. The effect of adding different levels of ascorbate in the presence of catechol upon oxygen uptake in homogenates from dormant tubers.

Since catechol is not likely to be the natural substrate for polyphenoloxidase in the tuber, dopa was used in the same manner as the study just described. It is evident that uptake of oxygen took place, and the quantity of oxygen absorbed is directly proportional to the quantity of ascorbic acid added (Figure 9A). Dopa oxidase activity was less when ascorbic acid concentration was high (Figure 9B), and this might show the probable reduction of dopa quinone which is formed by the oxidation of dopa. The oxidation and reduction might continue as long as ascorbic acid is available. When ascorbic acid quantity was high, oxygen uptake was greater and the color strength of hallochrome was less (Figure 9A, B). The homogenate which contained no ascorbic acid had a specific activity of 60, whereas ascorbate at the lowest concentration inhibited hallochrome formation by 33 per cent. The two higher levels of ascorbate inhibited hallochrome formation by 100 per cent (Figure 9B). An explanation of this is that dopa was dehydrogenated at its initial stage of oxidation by its oxidase which gave rise to an o–quinone, and this in turn was reduced by ascorbic acid which inhibited hallochrome formation. It appears that the quantity of the reduced form of ascorbate is the determining factor for the oxidation of dopa in the tubers of nut grass. It is concluded from these results that the presence of ascorbate probably prevents the formation of melanin in the tubers.

Perhaps catechol oxidation decreased during germination as a result of the oxidation of ascorbic acid by other ascorbic acid oxidases. This is possible, because dehydroascorbate level increased with an increase in ascorbate oxidation.

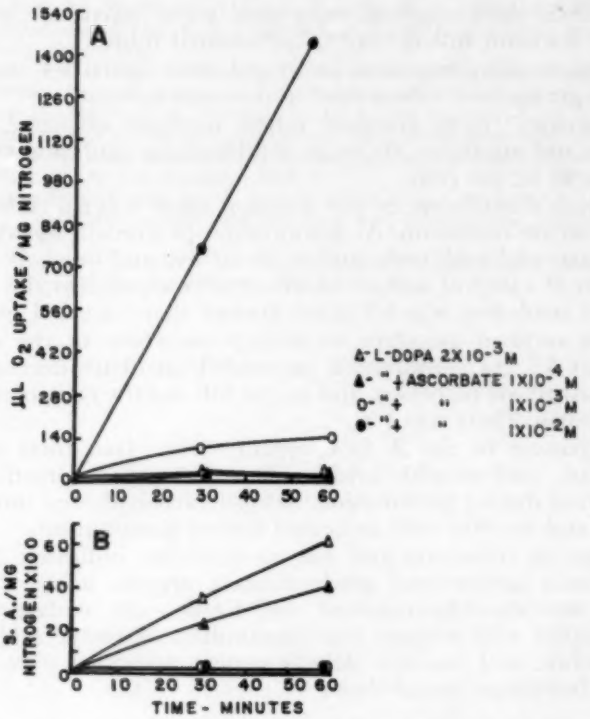

Figure 9. A—The influence of adding different levels of ascorbate in the presence of dopa upon oxygen uptake. B—The influence of ascorbate upon dopa oxidase activity in homogenates from dormant tubers.

SUMMARY

Studies on the metabolism of dormant and germinating tubers of nut grass showed the following results:

1. Dormant tubers showed an R. Q. of 2.1; however, oxygen consumption increased during germination and an R. Q. of unity was obtained in five and one-half days. Total gas exchange increased as germination proceeded.

2. Total organic acids, fats, and total nitrogen decreased in quantity from the time the tubers were dormant until about four days after germination. These materials increased in quantity from the 4th day of germination to the 8th day.

3. Citric, malic, and oxalic acid quantities remained unchanged during germination, but the quantity of citric was higher than malic and malic was higher than oxalic.

4. Soluble carbohydrates increased from dormancy to the 2nd day and then decreased until the 8th day of germination.

5. Isocitric dehydrogenase associated with "particles" was more active in dormant tubers than in germinated tubers.

6. Succinic dehydrogenase associated with "particles" was more active in germinated tubers than in dormant tubers.

7. "Particles" from dormant tubers oxidized isocitrate, a–ketoglutarate and succinate. Malonate inhibited the oxidation of a–ketoglutarate by 62 per cent.

8. Catechol oxidation by the dormant tuber was 5.9 times higher than ascorbate oxidation. As germination proceeded, ascorbate oxidation increased with time, and at about five and one-half days the oxidation of catechol and of ascorbate were equal. On the 8th day, ascorbate oxidation was 3.9 times greater than catechol oxidation.

9. The ratio of ascorbate to dehydroascorbate in the dormant tuber was 3.5. As germination proceeded ascorbate decreased and dehydroascorbate increased, and on the 8th day the ratio of ascorbate to dehydroascorbate was 1.5.

The changes in the R. Q.'s, organic acids, fats, total nitrogen, catecholase, and ascorbic acid oxidase occurred at practically the same period during germination. Soluble carbohydrates, total organic acids, and ascorbic acid decreased during germination.

Changes in substrates and enzyme activities indicated that dormant tubers metabolized predominately organic acids. The catecholase, isocitric dehydrogenase, and Krebs cycle oxidase activities were coupled with organic acid metabolism. Ascorbic acid oxidase and succinic and isocitric dehydrogenase activities were coupled with carbohydrate metabolism.

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The Metabolism of Nut Grass (*Cyperus rotundus* L.) III. Polyphenoloxidase in the Dormant Tuber

R. D. Palmer and W. K. Porter, Jr.[1]

The purpose here was to study fundamental aspects of polyphenoloxidase in dormant tubers.

Materials and Methods

The tubers used in these studies were grown as described previously (8).

I. Preparation of tissue components.

Tissue slices. Single dormant tubers were selected at random and sectioned on the sliding microtome. The sections were sliced at a thickness of 30 microns, and immediately immersed in distilled water. Before use in experiments the slices were blotted with paper towels.

Homogenates. The homogenates were prepared in the same buffer and by the same method as described previously (9).

II. Estimation of enzyme activities.

Oxygen uptake was measured by conventional Warburg methods (10).

Tyrosinase on washed tissue slices. Slices from dormant tubers were washed for 24 hours with running tap water. The sections were blotted with paper towels and 35 to 40 were floated in 2.5 ml of 0.05 M potassium phosphate buffer plus 0.02 M sucrose at pH 6.0 in Warburg flasks. A 0.5 ml aliquot of alkaline aqueous L-tyrosine was tipped from the side arm 10 minutes after equilibration. Water at the same pH as the L-tyrosine was tipped for the check treatment. Duplicate flasks were employed for each treatment and the test was duplicated. The final concentration of L-tyrosine is reported. The final pH was 6.3 and the temperature 25° C.

Activity and inhibition of catecholase. The general procedure followed was to use a 2 ml aliquot of a homogenate along with a 0.5 ml aliquot of inhibitors or catechol. The concentration of catechol and inhibitors is reported as final. Duplicate flasks were employed for each treatment and the test was duplicated. The catechol and inhibitors were prepared in the same buffer as the homogenates and adjusted to the proper pH. In studies in which the pH varied from 6.0, the homogenate was readjusted with either NaOH or H_2SO_4. After equilibrium in the water bath, the catechol in a single side arm or catechol and inhibitor in separate side arms of the vessels were tipped into the homogenate. Manometric readings were recorded at 10 minute intervals for one hour. In studies in which the pH was above 6.0, endogenous oxidation and autooxidation were determined and substracted from total oxidation. The data reported for these studies are of exogenous substrate oxidation.

The inhibitors, benzoic acid, thiourea and *p*-nitrophenol, were used at 25° C and pH of 6.5. Dieca (sodium-diethyldithiocarbamate) was used at pH 7.0 and 20° C. Catechol was used at the same pH as the inhibitors.

Activity of catecholase under oxygen levels. Tuber slices 30 microns thick were used, and 35 to 40 were floated in 2.5 ml of 0.05 M potassium phosphate plus 0.02 M sucrose of pH 6.0 in the main compartment of the Warburg flask. The flasks were flushed with 1 and 10 per cent oxygen in combination with nitrogen, and wtih 100 per cent oxygen. Approximately 1 liter of gas was flushed through each flask after equilibration. Following flushing the stopcocks of the flasks and manometers were closed and oxygen uptake was measured at 30° C. Duplicate flasks were used and the test was duplicated.

Estimation of tyrosinase, DL-dopa oxidase and o-cresolase. A 2 ml aliquot of the homogenate from dormant tubers was used. A 0.5 ml aliquot of DL-tyrosine, DL-dopa and p-cresol were each placed in the side arm of duplicate flasks.

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These compounds were tipped into the homogenate following equilibration. The final concentration of the compounds is reported. These substrates were prepared in the same buffer as the homogenate. DL–dopa and p–cresol were adjusted to pH 6.0. DL–tyrosine was brought into solution with NaOH. A 0.5 ml aliquot of the alkaline tyrosine was tipped into the homogenate. The final pH was 6.5.

III. Total nitrogen determination.

The tissue slices were taken from the Warburg flasks and washed to remove the buffer. The slices were dried, and total nitrogen in the slices and homogenates was determined by the method described previously (9).

RESULTS AND DISCUSSION

I. Tyrosinase on washed slices.

To examine the possibility that the amino acid, L–tyrosine, would be oxidized by washed tissue slices, this acid was tipped into flasks which contained washed slices at 30 microns. As may be seen in Figure 1, the $QO_2(N)$ was 3.2 times greater for the oxidation of

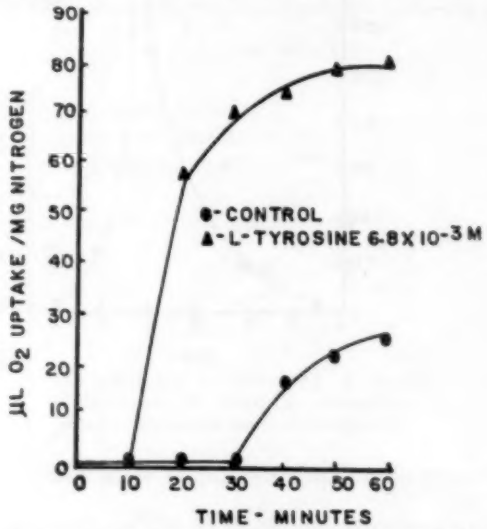

Figure 1. The oxidation of L–tyrosine by washed slices from dormant tubers.

L–tyrosine than the control. This indicates that tyrosinase was present in the tissue slices, and that perhaps a large portion of tyrosine was washed from the slices.

II. The effect of pH on catechol oxidation.

Catechol oxidation at pH 2.5 was practically nil, but at pH 4.0 the $QO_2(N)$ was 20 (Figure 2). At pH 6.5 the $QO_2(N)$ was 2.6 times higher than at pH 6.0, and at pH 7.0 the activity was about 20 $QO_2(N)$. No exogenous oxidation occurred at pH 8.0. These data point out that maximum oxidation of catechol in homogenates from dormant tubers took place at pH 6.5. It has been reported that

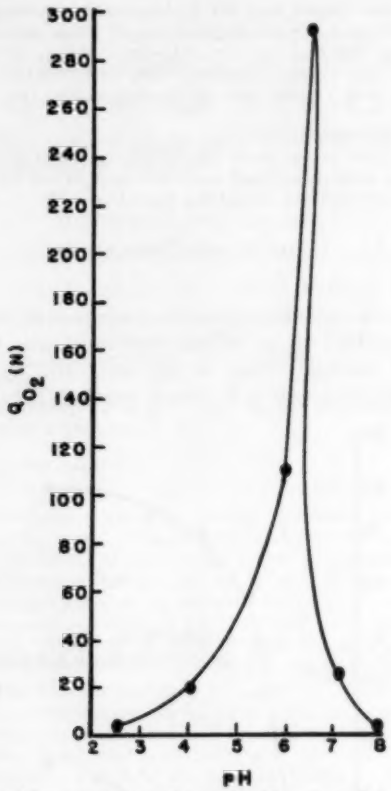

Figure 2. The effect of pH upon the oxidation of 0.024 M catechol in homogenates from dormant tubers.

maximum activity of polyphenoloxidase from plants was from pH 6.5 to 6.7 (3, 7).

To eliminate autoxidation, most of the studies with this enzyme from nut grass were conducted at pH 6.0.

III. Substrate studies.

Dawson and Tarpley (3) classified phenol oxidases from mushroom into monophenolase (creolase), and o–dihydric phenolase (catecholase) and used p–cresol and pyrocatechol, respectively, to determine these activities. Polyphenoloxidase is a general term applied here to the mono– and o–dihydric phenolases.

The most active substrate for the enzyme from nut grass was catechol. Oxidation of catechol by tissue slices of 30 microns thickness was markedly influenced by the oxygen concentration (Figure 3A). Catecholase was most active in 100 per cent oxygen. Palmer and Porter (8) have found that germination of dormant tubers was more rapid in 100 per cent oxygen. Thus, there is a correlation between

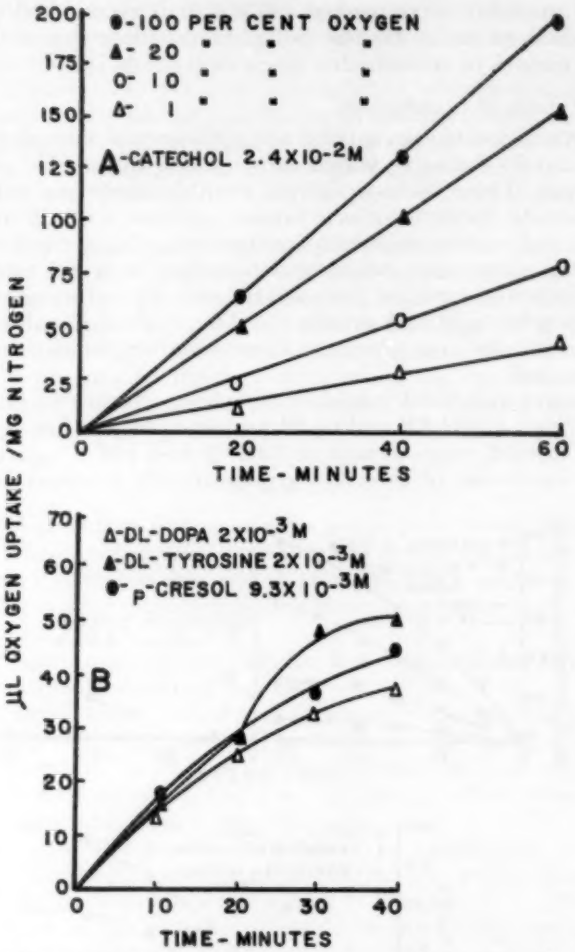

Figure 3. A—The influence of oxygen gradients upon the oxidation of catechol by tissue slices. B—The oxidation of DL-dopa, DL-tyrosine and *p*-cresol by homogenates from dormant tubers.

oxidation of catechol and germination under high oxygen levels. Palmer and Porter (9) also found that ascorbate was oxidized as a result of dopa oxidase in the tuber, and it is possible that this system might have served as the terminal enzyme in the dormant tuber. Probably isocitric dehydrogenase could have been coupled with tyrosinase which would make the aerobic system complete (9).

The monohydric-phenol, *p*-cresol and the phenolic amines, DL-tyrosine and DL-dopa, were oxidized in air considerably less than catechol (Figure 3A, B). DL-tyrosine was oxidized slightly more than DL-dopa, or *p*-cresol, but DL-tyrosine was used at pH 6.5, whereas

the other materials were used at pH 6.0. It is established that catechol is oxidized faster by the polyphenoloxidase system than the phenolic amines or monohydric phenols (1, 2, 3, 7).

IV. Inhibition of catecholase.

Most inhibition studies carried out with phenol-oxidizing enzymes have involved substances which form weakly dissociable complexes with copper. These include sodium cyanide, hydrogen sulfide, carbon monoxide, diethyldithiocarbamate, sodium azide, *p*–aminobenzoic acid, and various sulfhydryl compounds. Other types of inhibitors are (a) competitive inhibitors, including N–acetyl tyrosine and other substituted tyrosine derivatives, and (b) reducing substances such as ascorbic acid and sulfites (7). The inhibition values used in the discussion for nut grass are those obtained at the end of the measurements.

Oxalic acid at 0.02 M inhibited catecholase from nut grass tubers by 72 per cent at pH 4.0 and by 33 per cent pH 6.0 (Figure 4A). For the same period, benzoic acid at 0.02 M and pH 6.5 gave only 19 per cent inhibition of catecholase (Figure 4C). Carboxy acids were

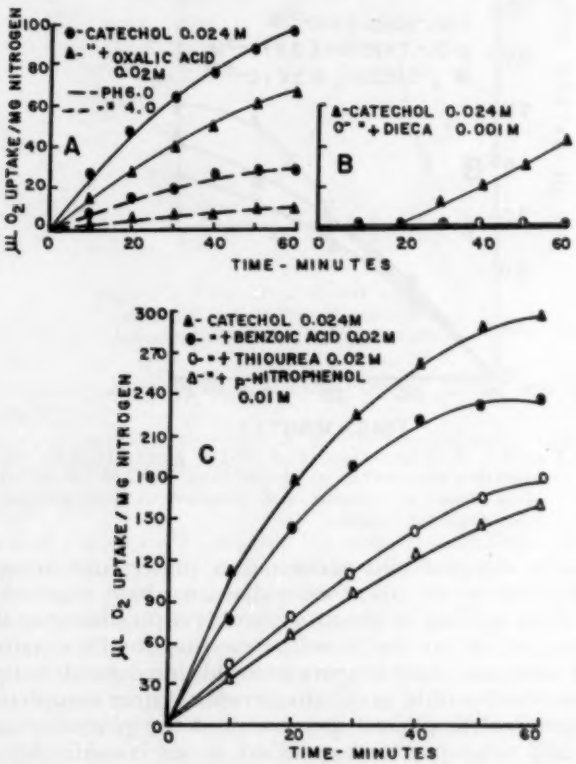

Figure 4. The effect of various inhibitors upon the oxidation of catechol in homogenates from dormant tubers.

shown by Krueger (6) to inhibit phenolase activity at low pH values. He attributed this to complexing of some active center of the enzyme by the carboxylic acid anion.

An inhibition of 47 per cent was obtained when *p*–nitrophenol at 0.01 M was used to inhibit catecholase in nut grass, whereas thiourea at 0.02 M inhibited catecholase by 41 per cent (Figure 4C). Bonner (2) attributed the inhibition of polyphenoloxidase by *p*–nitrocatechol and *p*–nitrophenol due to their resemblance to natural substrates.

Dieca at 0.001 M gave 100 per cent inhibition of catecholase (Figure 4B). James (4) reported that dieca appeared to be the most effective chelating agent for distinguishing copper enzymes from cytochrome oxidase. He reported that dieca at 0.002 M had practically no effect on cytochrome oxidase. James and Garton (5) obtained 100 per cent inhibition of ascorbic acid oxidase and polyphenoloxidase and 43 per cent inhibition of cytochrome oxidase with 0.001 M dieca at pH 7.0 and 20° C.

SUMMARY

Polyphenoloxidase was shown to operate in dormant nut grass tubers. The following data lend support to this conclusion.

1. When L–tyrosine was added to washed nut grass tissue slices the $QO_2(N)$ was 3.2 times greater for the oxidation of L–tyrosine than the oxidation for the control.

2. The optimum pH was 6.5 for the oxidation of catechol by homogenates from dormant tubers.

3. The most actively oxidized substrate was catechol in homogenates from dormant tubers. The oxidation of catechol by tissue slices was increased by oxygen concentrations from 1 to 100 per cent.

4. DL–tyrosine, DL–dopa, and *p*–cresol were oxidized about equally by the homogenates from dormant tubers.

5. Dieca gave 100 per cent inhibition of catecholase. The most effective inhibitor for catecholase at pH 6.5 was *p*–nitrophenol, followed by thiourea and benzoic acid.

6. Oxalic acid was twice as effective in inhibiting catecholase at pH 4.0 as at pH 6.0.

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The Metabolism of Nut Grass (*Cyperus rotundus* L.) IV. The Activities of Certain Enzymes From Tubers Treated With Amitrol

R. D. PALMER and W. K. PORTER[1]

SEVERAL studies have been conducted concerning the action of amitrol (3–amino–1,2,4–triazole) upon certain enzyme systems. The activity of catalase from treated potato plants and barley leaves (7), leaves of potato, barley, and sunflower, and rats (2) has been reported. In all cases, amitrol depressed the activity of catalase.

Heim et al. (2) have shown that peroxidase activity decreased slightly three hours after injection of amitrol into rate, but peroxidase returned to normal after 24 hours.

Herbert and Linck (3) found that amitrol increased respiration of leaf and rhizome sections of Canada thistle initially; however, the respiration decreased with time depending upon the concentration of amitrol and on the time interval following application. Phosphate levels in the Canada thistle tissue influenced the changes in all metabolism induced by amitrol treatment. McWhorter[2] found that chlorotic corn tissue obtained by soil application of amitrol exhibited a higher ascorbic acid oxidase activity than control tissue. Hollingsworth and Ennis (4) reported that amitrol was translocated from the leaves to the tubers of nut grass and inhibited their germination. In view of the above findings, it was considered worthwhile to examine the activity of some enzymes extracted from tubers previously treated with amitrol at concentrations which inhibited germination.

MATERIALS AND METHODS

I. Preparation of the plant material.

Nut grass grown as previously described (5) was used as a source of experimental materials.

II. Amitrol treatment.

Establishing the rate of amitrol to give shoot and root growth inhibition. Five grams of dormant tubers were placed in 125 ml Erlenmeyer flasks with four flasks being used for each treatment. To each flask, amitrol was applied at 10^5, 10^4, 10^3, 10^2, 10^1, 10^0 micrograms in 5 ml of complete Hoagland's nutrient solution at pH 5.7. The check contained no amitrol. The four flasks were connected in series with Tygon tubing and the system was flushed for two minutes with 100 per cent oxygen. The oxygen treatment was applied in order to speed germination (5). The tubing was sealed with screw clamps, and the experiment was kept in the laboratory. The system was flushed once every two days with 100 per cent oxygen. On the 8th day following treatment, shoot and root lengths were taken on 20 tubers from each treatment.

Amitrol treatment for catecholase, ascorbic acid oxidase, dopa oxidase, and peroxidase assay. Amitrol at 8 and 16 mg per 5 ml was used in Hoagland's nutrient solution at pH 5.7. These rates were in the range which gave root and

[1]Research Assistant and Associate Professor, respectively, Louisiana State University, Baton Rouge, Louisiana.

[2]McWhorter, C. G. Some effects of 3–amino–1,2,4–triazole on the respiratory activities of *Zea Mays* L. (A dissertation). Louisiana State University. 1958.

shoot growth inhibition as determined above. Oxygen treatment was the same as stated above, and there were 8 flasks per treatment. This study was conducted at 24 ± 2° C. and 310 foot candles of constant incandescent light. At the end of the 8th day, the tubers were utilized for the enzyme assays.

Amitrol treatment for catalase assay. The lower rate of amitrol in Hoagland's solution at pH 5.7 was used per 5 gm of tubers. The dormant tubers were placed in Petri plates containing the amitrol. The test was kept under variable room temperature, and constant fluorescent light at 53 foot candles in the laboratory. At the end of the 8th day of exposure, the tubers were prepared for catalase assay.

All tubers were washed in three liters of water before they were homogenized. This was done to remove the nutrient solution and amitrol.

III. Preparation of homogenates.

The homogenates were prepared as described previously (6). The buffer used to prepare homogenates for the activities of ascorbic acid oxidase, catecholase, dopa oxidase, and peroxidase was 0.05 M potassium phosphate at pH 6.0. The homogenates for catalase were prepared in 0.2 M potassium phosphate at pH 7.0.

IV. Estimation of enzyme activities.

Oxygen uptake was determined by using a Warburg respirometer and conventional methods described by Umbreit et al. (9).

Catecholase and ascorbic acid oxidase. The activities of catecholase and ascorbic acid oxidase were determined as previously described (6).

Dopa oxidase. The method of Bailey[a] was employed. Seven ml of buffer at pH 6.0, and 2 ml of 0.5 per cent (w/v in buffer) gelatin were added to a test tube and placed in a 25° C. constant temperature bath. Two ml of buffer containing 3.5 mg of L–dopa per ml were added followed by 1 ml of the homogenate. Adsorbance was determined with a 470 filter, and specific activity (S. A.) was computed as previously described (6).

Peroxidase. Peroxidase was determined by a modified method of Stutz (8). The buffer of the homogenate was adjusted from 0.05 M and pH 6.0 to 0.2 M and pH of 6.5 with potassium phosphate buffer. A 2 ml aliquot of the homogenate was added to a test tube, followed by 2.5 ml of freshly prepared pyrogallol. At zero time, 0.5 ml of hydrogen peroxide was pipetted into the tube. Optical density change was recorded at one minute intervals for five minutes by using a 420 filter at a temperature of 25° C. The final concentration for pyrogallol and hydrogen peroxide was 0.25 M and 0.006 M, respectively. These compounds were prepared in the buffer. The hydrogen peroxide was adjusted to pH 6.5 before adding to the buffer. A similar procedure was followed to measure autooxidation of pyrogallol and endogenous oxidation of the enzyme. For these determinations, the enzyme and pyrogallol were replaced by buffer. Specific activity was computed as previously described (6) with the exception that autooxidation of pyrogallol was substrated from total oxidation.

Catalase. A modified procedure of Appleman (1) was used for catalase determination. The hydrogen peroxide was prepared in the buffer after being adjusted to pH 7.0 with NaOH. A 2 ml aliquot of hydrogen peroxide was placed in the main compartment of the Warburg flask, and 1 ml of homogenate was tipped from the side arm. The final concentration of hydrogen peroxide was 1 M. Manometric readings were recorded at one minute intervals for four minutes. The temperature during the reaction was 20° C.

V. Determination of total nitrogen.

Total nitrogen in homogenates was determined by the method previously described (6).

RESULTS AND DISCUSSION

To establish the rate of amitrol which would inhibit germination, this compound was applied at logarithmic rates as shown in Figure

[a]Bailey, M. E. The biochemistry of melanin formation in shrimp (A dissertation). Louisiana State University. 1958.

1. It may be seen that amitrol at 10^4 micrograms inhibited both shoot and root growth.

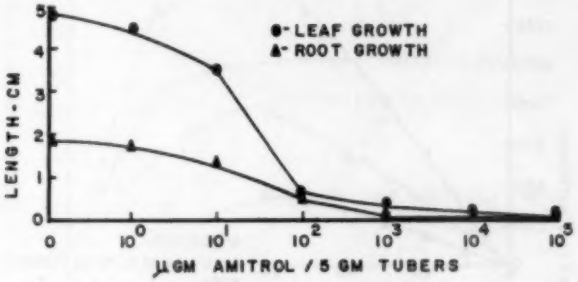

Figure 1. The effect of amitrol concentrations upon growth of the shoots and roots of nut grass tubers.

1. Enzyme activity determinations.

The activity used in the discussion is the total activity at the end of the measurements.

Ascorbic acid oxidase. The activity level of ascorbic acid oxidase in treated tubers was roughly proportional to the amitrol concentration. Homogenates from the germinated control oxidized ascorbate 1.9 and 4.4 times greater than homogenates obtained from tubers exposed to the low and high rates of amitrol, respectively (Figure 2A).

Catecholase. Catecholase was not predominant in the treated tubers, even though these tubers were not germinated (Figure 2B). Ascorbic acid oxidase was predominant, (Figure 2A), and this would indicate that similar changes of these enzymes occurred in the treated tubers as in a germinating control, because Palmer and Porter (6) found that dormant untreated tubers showed a higher level of catecholase than of ascorbic acid oxidase; as germination proceeded, ascorbic acid oxidase became greater than catecholase on the 6th day of germination. The ratio of ascorbic acid oxidase to catecholase was 3.9 in the germinated tuber cultured in water during 8 days of germination. The ratio of ascorbic acid oxidase to catecholase was 3.8 in the germinated tuber cultured in nutrient solution at pH 5.7 for the same period (Figure 2A, B). However, ascorbic acid oxidase was 6.9 times higher in tubers cultured in nutrient solution (Figure 2A), than in germinated tubers in water. It is interesting that the ratios were quantitatively the same. The reason that activities of these enzymes were higher in tubers cultured in nutrient solution than in water probably was due to light intensity and quality, temperature, nutrition, higher oxygen gradient, and rate of growth, or stage of growth at the end of the experimental period. The tubers cultured in nutrient solution were germinated in the presence of 95 to 100 per cent oxygen. The temperature was 24 ± 2° C. and the light was 310 foot candles of constant incandescent, whereas 53 foot candles of constant fluorescent light was used for tubers cultured in water. The temp erature was variable under laboratory conditions. Rate of growth probably was the most important contributing fac-

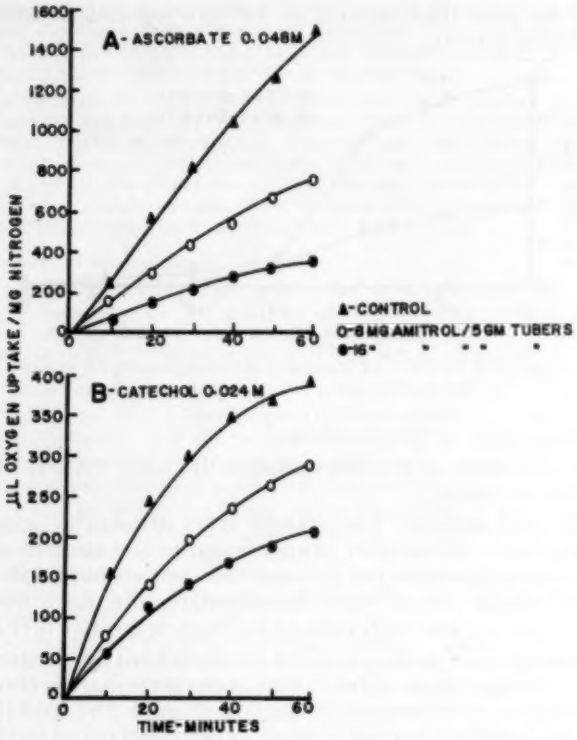

Figure 2. The oxidation of A—ascorbate, and B—catechol, by homogenates prepared from tubers which were exposed to amitrol treatment for 8 days.

tor, though no data are available on dry weights for comparison of tubers grown under these two conditions. Palmer and Porter (6) found that as germination proceeded and total fresh weight increased, an increase in activity of ascorbic acid oxidase occurred. In initial experiments, tubers which had germinated for 7 days in water showed an ascorbic acid oxidase activity of 248 microliters of oxygen per hour per gm of fresh weight, whereas tubers which had germinated for 17 days showed activity of 2200 microliters of oxygen on the same basis.

Catechol added to homogenates from control tissue was oxidized 1.4 and 1.9 times greater than the oxidation by homogenates from tubers treated with the low and high rates of amitrol, respectively (Figure 2B).

Dopa oxidase. The dopa oxidase activity was 1.5 times greater in the dormant tuber than in the germinated tuber (Figure 3). The level of activity in the dormant tuber was 1.8 and 3.8 times higher than the activity for tubers treated with the low and high rates of amitrol, respectively. Germination inactivated dopa oxidase some-

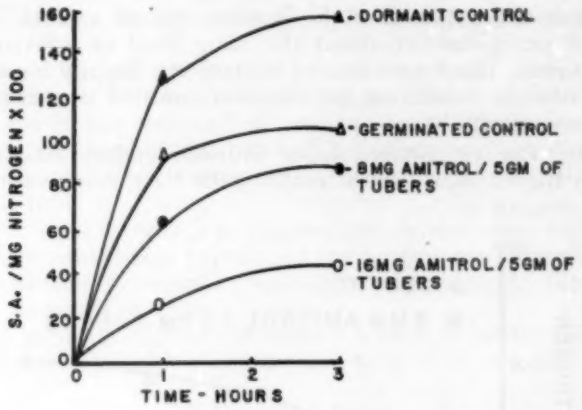

Figure 3. Dopa oxidase activity in homogenates from a dormant control, a germinated control, and in homogenates from tubers which were exposed to amitrol treatments for 8 days.

what, but amitrol treatment inactivated dopa oxidase even more than germination.

Peroxidase. The peroxidase level was 2.5 times higher in the germinated tuber than in the dormant tuber (Figure 4). It is ap-

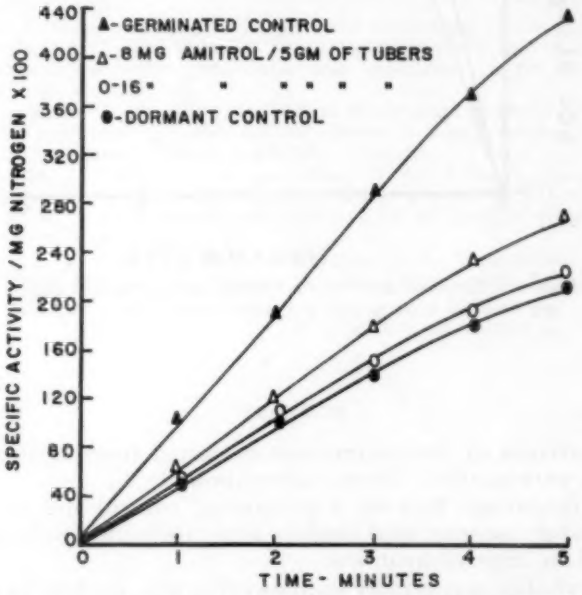

Figure 4. Peroxidase activity in homogenates prepared from a dormant control, a germinated control and in homogenates prepared from tubers exposed to amitrol treatments for 8 days.

parent from these data that the highest rate of amitrol held the activity of peroxidase at about the same level of activity as the dormant tuber. The lowest rate of amitrol was slightly less effective. Consequently, in inhibiting germination, amitrol treatment inhibited peroxidase activity.

Catalase. The germinated tuber showed catalase activity to be 18.5 times higher than tubers treated with 8 mg of amitrol per 5 g of tubers (Figure 5).

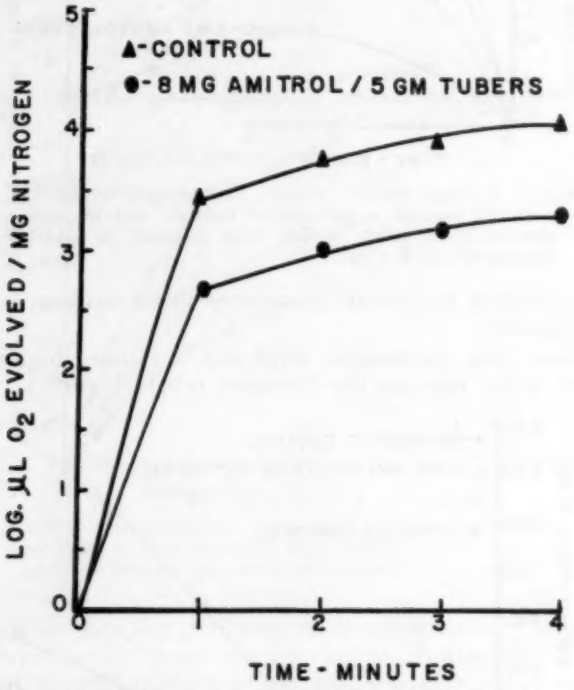

Figure 5. Catalase activity in homogenates prepared from germinated tubers, and prepared from tubers exposed to amitrol for 8 days.

SUMMARY

The activities of certain enzymes extracted from amitrol-treated nut grass were studied. These studies showed:

1. In comparison between a germinated control and an amitrol-treated tuber, ascorbic acid oxidase and catecholase were markedly depressed by amitrol treatment.

2. Catecholase was greater than ascorbic acid oxidase in dormant untreated tubers. Ascorbic acid oxidase was higher than catecholase in the treated tubers and the germinated tubers, but the treated tubers did not germinate. Thus, similar changes in these enzymes

occurred in the treated tubers and in the germinated untreated tubers.

3. The activity of dopa oxidase in the dormant tuber was high. Dopa oxidase activity in the tubers treated with 8 mg and 16 mg of amitrol was 20 per cent and 60 per cent less, respectively, than the activity in the germinated tuber. The treated tubers did not germinate, but did not show as active dopa oxidase as the dormant untreated tuber.

4. Peroxidase activity was approximately the same for the dormant tubers and tubers exposed to the highest rate of amitrol. The germinated tubers showed an activity of peroxidase 2.5 times higher than the dormant or treated tubers.

5. The germinated tuber exhibited a catalase activity 18.5 times higher than tubers which were treated with 8 mg of amitrol.

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COMMUNICATION

Donomen: A Pesticides Nomenclature System

E. C. S. LITTLE[1]

MOST people connected with the making, marketing and using of pesticides agree that the naming for common use of the stream of new chemicals could be better organised, especially on an international basis.

The chemical names of the new substances, nearly all organic and usually complex, are usually so cumbersome and incomprehensible to the uninitiated that they are quite useless for everyday speech and are a serious hindrance even to technicians familiar with them. The usual alternatives of code numbers or alphabetical abbreviations are not much better.

It needs a scientific approach as to how this international problem is to be solved, but as yet there is no obvious sign of much energy being directed towards this highly important end. This needs emphasising because it may be overlooked even by those who know that the progress of biological sciences would long ago have been halted if a naming system had not been developed for the vast numbers of different organisms under study.

The total number of pesticides being developed to deal with organisms of agricultural, industrial or medical importance has not yet become really large but a state has already been reached where confusion is impeding progress.

What, therefore, is wanted? The following points list the essential requirements for common names with which most people would agree:

(a) Names, for common use, issued as labels for all new chemicals before they are announced to the public.
(b) Names which are easy to say without ambiguous alternative pronunciations.
(c) Names which are easy to spell and are phonetic.
(d) Names which are internationally acceptable to all countries using, or familiar with, the Roman alphabet.
(e) Names which are of a class unlike other common words and thus are not likely to be confused with everyday language.
(f) Names which are easily coined by simple systematic rules.

It is not necessary to strive for some similarity between the common name and the full chemical one because this is difficult, complicates the issue, and seldom is effective. With usage the name, whatever it is, becomes identified with the chemical. People other than technicians are not interested in chemical structure. They only want a handle by which the substance can be linked to its properties and by which proprietary products can be judged.

The method which is now described is an attempt to meet these requirements. It is called Donomen. It is based on the principle that

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all pesticide common names should be tri-syllabic words. Such words when simple, come easily to the tongue, are long enough for clarity and short enough for simplicity.

They are coined by the following system:

1. The words will all consist of 4 consonants and 3 vowels and thus be 7 letters long.
2. The words will begin with a consonant and have vowels and consonants alternating until the last consonant which finishes the word e.g. TENIBOL. The words would be pronounced with all vowels short and the emphasis on the first 3 letters and the next two pairs equally, i.e. ten-ib-ol.
3. Any consonants which are likely to cause spelling errors are excluded. Such consonants are:—

   Q confusable with K
   C confusable with K or S
   Y confusable with I
   X confusable with KS
4. By these rules consonant conjunctions such as TR or ST or BL and double vowels are also excluded.

There are thus 17 consonants and 5 vowels available which would give 17 x 17 x 17 x 17 x 5 x 5 x 5 combinations = 10½ million. There are thus enough names available between BABABAB and ZUZUZUZ to cope with all the output of chemists virtually for ever.

Critics of such a scheme might use these words as examples of ridiculous names which can be coined. This is true if by ridiculous is meant out of the ordinary. But the point of this system is that the coined names will be different from ordinary speech. They will be a new language—the language of pesticide nomenclature. With 10 million to choose from the more extravagantly peculiar words need not be chosen and there are ways by which this selection can be assisted by additional rules.

However it is interesting if, as an experiment, an attempt is made to imagine that an exceptionally peculiar word is already established. Suppose bababab to be the name of a well known insecticide. After a number of repetitions of the word as part, say, of imaginary recommendations it soon begins to lose its strangeness and becomes just another name—in style not so very different from DDT.

Donomen words are already among the most successful in pesticide nomenclature. They come easily to the tongue, they are seldom spelled incorrectly and do not promote much argument about pronunciation.

Examples are simazin, dinoseb, dalapon, (weed killers), demiton, (insecticide) and fumarin (rodenticide).

For coining Donomen words a machine could be set up to type them out mechanically. A number of rules could be fed into the machine to exclude words which are particularly grotesque or which present any difficulties in pronunciation.

Such rules could include:

(a) The vowel U should not precede H, R or W (when it tends to be pronounced long as in monuron).

(b) The consonants H and W present difficulties and should only be used in the initial position.
(c) Any one consonant should not appear more than 3 times in one word and then not consecutively.
(d) Any one vowel should not appear more than twice in any one word. These modifications bring the words available down to about 8 million.
(e) Words should be printed at random but a memory store kept so that they are not repeated and none left out.

By this method there would be no longer grounds for those, such as the Terminology Committee of the Weed Society of America (1) who say, "It is difficult if not impossible to coin common names for herbicidal chemicals until they have reached the commercial development stage." A similar statement is made by the British Standards Institution (2).

To get pesticide nomenclature efficiently established international agreement is needed on the following lines:

1. That a standing committee of the United Nations (arranged, say, by F.A.O.) be appointed for nomenclature.
2. That a system of coining names based on the principle suggested here (or a better one) be adopted, mechanical aids to production of the names being used.
3. That before any new chemical is announced in any way by public lecture or article a common name has to be applied for; this name to be used in conjunction with the chemical name until the common name becomes fully established.
4. If the chemical fails to become commercially important it does not affect its common name which remains on the records in case the chemical should ever be revived.
5. If secrecy is necessary for those who wish to obtain patents, then, by permission, the common name, unconnected with its chemical description, could be used until such time as protection has been obtained. This will avoid the confusing coding systems often submitted by commercial firms who announce new chemicals.
6. It would be necessary for general agreement for commercial firms to avoid Donomen words for trade names (there are only a few in use as yet). It would be desirable if firms, after using whatever trade name they considered appropriate would then, as a general rule, insert in the label, "Contains....." giving the Donomen common name.

There are probably only a few dozen Donomen type words in English. Some examples are: general, develop, janitor, monitor, regular, seminar, benison, tabular, popular and venison; and a number ending in -ed, such as defined, damaged, desired, related, which are not pronounced according to Donomen rules.

The only words that spring to mind from foreign language are kamerad and matelot! It would be interesting to list how often this word form is used in different languages.

Here are a few Donomen words, coined at random to illustrate the

style they take: putalet, rodinef, duborit, miperos, positon, vedikor, mitinad, burijak, gatelas, benidin etc. etc.

Few Donomen words mean anything and it would be rare for any commercial firm to have to complain because they had been issued with a word like "mukinit" or worse still "tisadud"! Play on Donomen words in spite of their simplicity is difficult, especially if they are pronounced according to rule and therefore they should come to mean, as they are selected, one thing only—an easy name to describe a complex chemical.

There are ample words available so that combinations which had any significance in any language could be rejected.

The suggestions made for the Donomen system may have some features which are not acceptable to everyone. Ideas as to how the system could be improved would be welcomed. If from discussion and suggestion a fully workable nomenclature system were to be created and implemented then the purpose of this communication will have been achieved.

LITERATURE CITED

1. Report of the Terminology Committee, Weed Society of America. Weeds 6:71. 1958.
2. British Standards Institution "Recommended Common Names for Pesticides." 1957.

This communication was referred to the Terminology Committee of the Weed Society of America. All members agreed that it should be published as a stimulant to discussion. It is ironic that two of the few Donomen-form names for herbicides proposed in the past year are being objected to by other companies as being too similar to previous trade-marked Donomen-form names they are now using. This underscores the fact that the 2nd paragraph on page 520 may be too optimistic. As one committee member says "The cost of developing a coined common name for a pesticidal chemical continues to be very high and the procedure is difficult. It appears that it will be difficult to coin common names for herbicides before they reach the commercial stage of development."

Other Committee comments: "The use of tri-syllabic system as suggested gives rise to a great number of words, most of which are out of the ordinary and need practice to become accustomed to speaking. From a commercial point of view this is a great handicap. A name which has a spontaneous appeal is the one most desired. It is a big job to educate the user to a new language."

"The need for new names is not great, as there are relatively few new pesticides introduced each year. The most objectionable part is the suggestion that names are to be assigned by some agency and the originator has no choice. The most favorable part for the tri-syllabic system is that it may be used as a guide by manufacturers so that some general plan may be followed. This usage should be voluntary and the name chosen by the originator of the pesticide."

This subject is being discussed elsewhere as well. Comments may be sent to WEEDS and will be forwarded to Mr. Little. EDITOR.

News and Notes

The officers and meeting dates of the Weed Society of America and the various weed conferences are as follows:

Weed Society of America:

President, A. S. Crafts, University of California, Davis.
Vice-President, K. P. Buchholtz, University of Wisconsin, Madison.
Secretary, W. C. Shaw, Crops Research Division, ARS, USDA, Beltsville, Maryland.
Treasurer and Business Manager, W. C. Jacob, University of Illinois, Urbana.
Editor, C. J. Willard, Ohio State University, Columbus 10.
Next meeting—February 22 to 25, 1960, at the Cosmopolitan Hotel, Denver, Colorado. The Western Weed Control Conference is host for this meeting.

Western Weed Control Conference:

President, R. A. Fosse, Amchem Products, Inc., Denver, Colorado.
Vice-President, W. R. Furtick, Oregon State College, Corvallis.
Secretary-Treasurer, E. E. Heikes, Montana State College, Bozeman.
Next meeting, February 22 to 25, 1960, Cosmopolitan Hotel, Denver, Colorado, where the Conference is host to the Weed Society of America.

Southern Weed Conference:

President, V. S. Searcy, Alabama Polytechnic Institute, Auburn.
Vice-President, Robert Darrow, Texas A and M College, College Station.
Secretary-Treasurer, Walter K. Porter, Jr., Louisiana State University, Baton Rouge.
Next meeting, January 20, 21 and 22, 1960, at Biloxi, Mississippi.

Northeastern Weed Control Conference:

President, L. G. Utter, The Diamond Alkali Co., Painesville, Ohio.
Vice-President, E. M. Rahn, University of Delaware.
Secretary-Treasurer, D. A. Schallock, Rutgers University, New Brunswick, New Jersey.
Next meeting, January 6, 7, and 8, 1960, at Hotel New Yorker, New York city.

North Central Weed Control Conference:

President, J. Roe Foster, Canadian Department of Agriculture, Indian Head, Saskatchewan.
Vice-President, L. G. Holm, University of Wisconsin, Madison.
Secretary-Treasurer, John D. Furrer, University of Nebraska, Keim Hall, Lincoln.
Next meeting, December 8, 9, and 10, at the Royal Alexandra Hotel, Winnipeg, Manitoba. This is a joint meeting with the Western Canadian Weed Control Conference.

Professor G. E. Blackman, Department of Agriculture, Oxford University, Oxford, England, is spending much of the summer of 1959 visiting research stations, chemical companies, and scientific meetings in the United States and Canada.

T. J. Sheets, Crops Research Division, ARS, USDA, has been transferred from Davis, California, to Stoneville, Mississippi, where he will work with Drs. J. T. Holstun, Jr. and C. G. McWhorter on the cooperative weed research program for cotton. He completed the Ph.D. in plant physiology at the University of California, Davis, in June, 1959.

The Fifth Annual Cornell Weed Day was held July 21 and 22 with 153 College and Industry personnel attending. A smoker and discussion session on the evening of the 21st covered granular herbicides. The field tour on July 22nd covered plots on small grains, forage legumes, perennial weed control, life history studies of perennial weeds and vegetable crops.

Dr. M. M. Schreiber, Research Agronomist of the Crops Protection Research Branch, ARS-USDA, has been transferred from Cornell University to Purdue University.

Dr. H. Wayne Hilton recently became Principal Chemist at the Experiment Station of the Hawaiian Sugar Planters' Association in Honolulu. He replaces Dr. Francis E. Hance who retired at the end of last year. Dr. Hance had been Principal Chemist at the above Station since 1926. During this time he was active in studies of herbicide chemistry. His main research was on oil emulsions activated with pentachlorophenol or its sodium salt. He holds two patents on herbicides and corrosion inhibition as it relates to herbicides and other agricultural chemicals. Dr. Hilton was previously engaged in by-products research at the Experiment Station, Hawaiian Sugar Planters' Association. As Principal Chemist he will be in charge of herbicide formulation for the Hawaiian sugar industry. Dr. Hilton received his doctorate in Organic Chemistry at Ohio State University in 1952.

Dr. Kenneth A. Sund joined the Chemistry staff of the Experiment Station, Hawaiian Sugar Planters' Association July 1, 1959 as Senior Chemist. Dr. Sund was engaged in herbicide research for one of the large chemical companies until 1956 when he went to the University of Massachusetts to work for his doctorate. He received his degree in June of this year. Dr. Sund will be engaged in herbicide research and formulation with Dr. Hilton.

Dr. Noel S. Hanson recently became coordinator of weed control research for the Experiment Station, Hawaiian Sugar Planters' Association. This covers both fundamental and applied research in the Hawaiian sugar industry. Dr. Hanson has been in charge of field studies on weed control in sugar cane in Hawaii since 1948. He received his doctorate from the University of Nebraska in 1955 following a year's leave of absence for study from the above Station.

Dr. Francis E. Hance was recognized by his colleagues at a recent meeting of the Hawaii Weed Conference. An Honorary Membership in the HWC was conferred on Dr. Hance for "signal service to his profession as a chemist, as a fine gentleman and a good citizen". Others who have been given Honorary Membership in HWC in the past are John C. Ripperton, now retired Head of the Agronomy Department at the University of Hawaii and James P. Michels, now retired Maintenance Engineer at the Hawaiian Electric Company in Honolulu.

The 1959 Amchem Weed Field Day was held at the company's Ambler, Pa., research farm on July 29. During the day's tour, 57 weed workers from 8 nearby states were shown greenhouse, field secondary and woody plant screening operations, soil sterilant, Canada thistle and nutgrass control projects, and vegetable, corn, soybean and turfgrass weed control plots. After a buffet lunch, Professor G. E. Blackman of Oxford University explained the organization of weed control research, regulations and herbicide use in England.

Membership List of Weed Society of America

July 1, 1959

ALABAMA

Harold Benford, Progressive Farmer, Birmingham 2
Donald E. Davis, Dept. of Bot. Pl. Path., Alabama Poly. Tech., Auburn
Lester H. Hartwig, Amchem Products Inc., Echo Ridge Farm, Union Springs
Sam T. Jones, Horticulture Dept., A.P.I., Auburn
John M. Lawrence, Dept. of Zool. & Ent., Alabama Poly. Tech., Auburn
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BIBLIOGRAPHY OF WEED INVESTIGATIONS
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Agricultural Research Service
Crops Research Division
Weed Investigations Section

This bibliography of current literature on weeds and their control is issued as a service to those engaged in weed research in the United States and other countries. In issuing this bibliography the Crops Research Division, Agricultural Research Service, U.S.D.A. does not assume responsibility for the subject matter cited.

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